VOLUME 44 NOVEMBER 1954 NUMBER 11

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

UNIVERSITY OF MICHIGAN
NOV 11 1954
SCIENCE LIBRARY

CONTENTS

Relation of hydrogen-ion concentration of nutrient solution to Phytophthora root rot of avocado seedlings ..... Frank T. Bingham and George A. Zentmyer 611

Effect of temperature and humidity on the grape powdery mildew fungus ..... Charles J. Delp 615

Bacterial stem rot and leaf spot of Pelargonium ..... Donald E. Munnecke 627

Swedes naturally infected with two viruses ..... G. H. Berkeley and J. H. Tremaine 632

Hendersonula toruloidea Nattrass on citrus in California ..... E. C. Calavan and J. M. Wallace 635

Inheritance of resistance to infection by tobacco-mosaic virus in tomato ..... Francis O. Holmes 640

Isolation and identification of L-pipecolic acid from Western-X diseased peach leaves ..... Theodor O. Diener and Charles A. Dekker 643

Control of bacterial wilt of chrysanthemums with streptomycin ..... R. S. Robison, R. L. Starkey, and O. W. Davidson 646

A study of cane and crown galls on Vancouver Island and a comparison of the causal organisms ..... W. E. McKeen 651

The response of ten clonal lines of Bromus inermis to infection by Selenophoma bromigena ..... Hugh Wintersteen 655

Host pathogen relations in powdery mildew of barley. I. Histology of tissue reactions ..... N. H. White and E. P. Baker 657

Wilt resistant mutations in the Cayenne variety of pineapple ..... J. L. Collins and Walter Carter 662

Subscription, $10.00 per year

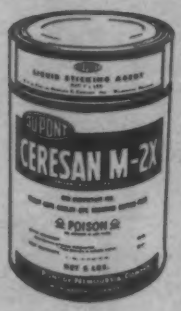
Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

Du Pont offers . . .

Superior Seed Protection

. . . for every type of treating equipment



READY MIX. Du Pont Liquid 244 is the new disinfectant made specially for ready-mix (fully automatic) treaters. Used according to directions, Liquid 244 protects wheat, oats, barley, rye and flax against seed- and soil-borne diseases. Like famous "Ceresan" products, Liquid 244 contains volatile mercury, whose vapor penetrates cracks and crevices of the seed to kill disease organisms. It's dustless at all times.

SLURRY. Du Pont Liquid 364 is the new product for slurry treater operators who prefer a liquid. It prevents seed- and soil-borne diseases of wheat and small grains because penetrating mercury vapors get into cracks and crevices. Both these liquid formulations contain two types of mercury—for volatility and for long-lasting effectiveness.

"Ceresan" M-2X Seed Disinfectant treats twice as much seed per pound as "Ceresan" M, means less handling for treaters. Also, "Ceresan" M-2X is dustless . . . easier to work with. This reliable seed protection is low in cost and has a proven record in the establishment of healthy stands of grain. A special sticking agent is attached to each 5-lb. can of "Ceresan" M-2X to assure more complete coverage and eliminate dust during treating and planting.

DRY. "Ceresan" M seed disinfectant is known as the standard . . . has helped farmers get better grain yields for over 20 years. It's always used as the check by investigators who are testing new products. The original mercurial seed treatment, "Ceresan" is best for customers who use the dry method of treating.

"CERESAN" IS A REGISTERED TRADEMARK OF E. I. DU PONT DE NEMOURS & CO. (INC.)

DU PONT
REG U.S. PAT. OFF.

BETTER THINGS FOR BETTER LIVING . . . THROUGH CHEMISTRY

On all chemicals always follow directions for application. Where warning or caution statements on use of product are given, read them carefully.

Seed Disinfectants

E. I. du Pont de Nemours & Co. (Inc.)
"Semesan" Products, Wilmington 98, Delaware

PHYTOPATHOLOGY

An International Journal

Official Organ of The American Phytopathological Society

EDITORS

W. C. PRICE, Editor-in-Chief, Citrus Experiment Station, Lake Alfred, Florida

C. S. HOLTON, State College of Washington, Pullman, Washington

L. M. BLACK, Department of Botany, University of Illinois, Urbana, Illinois

S. E. A. MCCALLAN, Boyce Thompson Institute, Yonkers 3, N. Y.

ASSOCIATE EDITORS

W. B. ALLINGTON, University of Nebraska, Lincoln, Nebraska

EDWARD O. OLSON, Citrus Rootstock Investigations, Box 144, Weslaco, Texas

A. W. DIMOCK, Cornell University, Ithaca, New York

GLENN S. POUND, University of Wisconsin, Madison, Wisconsin

M. T. HILBORN, Agricultural Experiment Station, Orono, Maine

MORTIMER P. STARR, University of California, Davis, California

W. J. HOOKER, Iowa State College, Ames, Iowa

ROGER U. SWINGLE, P. O. Box 70, 5076 North High Street, Worthington, Ohio

W. F. JEFFERS, College Heights, Hyattsville, Maryland

ALBERT L. TAYLOR, Plant Industry Station, Beltsville, Maryland

ARTHUR KELMAN, North Carolina State College, Raleigh, North Carolina

C. E. YARWOOD, University of California, Berkeley, California

BUSINESS MANAGEMENT

SAUL RICH, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

L. J. ALEXANDER, Advertising Manager, Agricultural Experiment Station, Wooster, Ohio.

NOTICES

Send communications regarding subscriptions and back numbers to SAUL RICH, Business Manager, 32nd St. and Elm Ave., Baltimore, Md., or P. O. Drawer 1106, New Haven 4, Connecticut.

Subscription price. $10.00 per year, domestic and foreign. Single current numbers (within 60 days of publication), $1.00. The journal has been issued monthly beginning with Volume 8. **Separate Numbers** may be purchased from back volumes at $1.00 per copy. Complete unbound volumes, 1 through 7, when available, $6.00 each; 8 through 41, as available, $10.00 each. **Thirty-Year Index** (Vols. 1–30, 1911–1940), bound: $7.50, domestic and foreign. **Ten-Year Index** (Vols. 31–40, 1941–1950), paper cover, $3.50. **Lost Numbers.** Claims for copies (domestic subscriptions) lost in the mails must be received within 60 days from the date of issue (Jan. 15 and monthly thereafter); otherwise the usual charge of $1.00 will be made for replacements. Deliveries overseas are not guaranteed and foreign mailings are made at the subscriber's risk. **Separates.** No gratis copies supplied. Quotation is sent with proof.

Advertisements. Rates may be secured from the Advertising Manager. No indorsement of any statement of claims made in advertisements is assumed by this Journal or by The American Phytopathological Society.

Manuscripts and editorial correspondence should be sent to the Editor-in-Chief. At least 1 of the authors must be a member of the American Phytopathological Society. Type on only 1 side of sheet; double-space all parts of manuscript, including footnotes, citations, and legends. For papers longer than 1 printed page, the article should begin with a brief, concise summary. Number footnotes consecutively with arabic numerals, except in tables where lower case letters are used. Give each table a heading stating its contents. Make references to literature as footnotes only when 5 or fewer citations are given. Name and address of the institution where the work was done should precede "Literature Cited." List references alphabetically in "Literature Cited" and number consecutively throughout text as well as in list of citations. Until further notice, authors are advised to read "The preparation of manuscripts for Phytopathology" by A. J. Riker, published in PHYTOPATHOLOGY 36: 953-977, 1946, as well as current issues of PHYTOPATHOLOGY.

Illustrations must be limited in number and should be sharp and clear. One-half the cost of engraving in excess of $16.00, the approximate cost of a single full page cut or of two 3 in. x 4½ in. cuts, are charged to the author. No charge is made for line drawings that substitute for tables unless drawings and tables together exceed 30 per cent of the length of the article. Illustrations are run as text figures designated by arabic numerals. Units of a composite illustration are designated by letters. Arrange full-page figures to conform to journal proportions (6⅛ x 8½ in.), making due allowance for reductions. Specific reference to each illustration must be made in the text, and each must be accompanied by an adequate legend, typed double-space on a separate paper and not on the illustration.

The responsibility for statements, whether of fact or opinion, rests entirely with the writers thereof.

The American Phytopathological Society

FOUNDED 1908. INCORPORATED 1915.

THE A. P. S. COUNCIL

President—George F. Weber, University of Florida, Gainesville, Florida
Vice-President—Helen Hart, University of Minnesota, University Farm, St. Paul 1, Minnesota
Secretary—Glenn S. Pound, University of Wisconsin, Madison 6, Wisconsin
Treasurer—Saul Rich, Connecticut Agricultural Experiment Station, P. O. Drawer 1106, New Haven 4, Conn.
Editor-in-Chief—W. C. Price, Citrus Experiment Station, Lake Alfred, Florida
President-Elect—J. H. Jensen, Iowa State College, Ames, Iowa.
Retiring President—J. G. Dickson, University of Wisconsin, Madison 6, Wisconsin

Councilors:

S. J. P. Chilton, Louisiana State University, Baton Rouge, Louisiana
George W. Fischer, State College of Washington, Pullman, Washington
William B. Hewitt, University of California at Davis, California
F. O. Holmes, The Rockefeller Institute for Medical Research, New York 21, New York
Paul R. Miller, Plant Industry Station, Beltsville, Maryland
J. R. Shay, Purdue University, Lafayette, Indiana
W. J. Zaumeyer, Plant Industry Station, Beltsville, Maryland

DIVISIONS OF THE SOCIETY

Northeastern: J. S. Tidd, Associated Seed Growers, Inc., P. O. Box 406, New Haven 2, Connecticut, *Pres.;* A. B. Burrell, Cornell University, Ithaca, New York, *Vice Pres.;* A. C. Tarjan, University of Rhode Island, Kingston, R. I., *Sec.-Treas.*

North Central: Donald Cation, Michigan State College, East Lansing, Michigan, Pres.; C. O. Johnson, Kansas State College, Manhattan, Kansas, Vice-Pres.; M. B. Linn, University of Illinois, Urbana, Illinois, Sec.-Treas.

Pacific: Gilbert L. Stout, Bureau of Plant Pathology, Sacramento, California, *Pres.;* George A. Zentmyer, Citrus Experiment Station, Riverside, California, *Vice-Pres.;* Roy A. Young, Oregon State College, Corvallis, Oregon, *Sec.-Treas.*

Potomac: J. W. Heuberger, University of Delaware, Newark, Delaware, *Pres.;* H. Rex Thomas, Plant Industry Station, Beltsville, Maryland, *Vice-Pres.;* L. O. Weaver, University of Maryland, College Park, Maryland, *Sec.-Treas.*

Southern: J. H. Miller, University of Georgia, Athens, Georgia, *Pres.;* T. W. Graham, Pee Dee Experiment Station, Florence, South Carolina, *Vice-Pres.;* G. B. Lucas, Plant Pathology, North Carolina State College, Raleigh, North Carolina, *Sec.-Treas.*

SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Virginia.
Butler County Mushroom Farm, Inc., West Winfield, Pennsylvania
California Packing Corporation, Swedesboro, New Jersey
Campbell Soup Company, Agricultural Research Department, Riverton, New Jersey
The Dow Chemical Company, Midland, Michigan
E. I. du Pont de Nemours & Company, Incorporated, Wilmington 98, Delaware
Ferry-Morse Seed Company, P. O. Box 778, Detroit 31, Michigan
Freeport Sulphur Company, New Orleans 5, Louisiana
H. J. Heinz Company, Pittsburgh 30, Pennsylvania
Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.
Mathieson Chemical Corporation, Mathieson Building Baltimore, Maryland
Pennsylvania Salt Manufacturing Company, Box 4388, Chestnut Hill P. O., Philadelphia 18, Pa.
Rohm & Haas Company, Washington Square, Philadelphia 5, Pennsylvania
Shell Chemical Corporation, Julius Hyman & Company Division, P. O. Box 2171, Denver 1, Colorado
Tennessee Corporation, 619-27 Grant Building, Atlanta 1, Georgia
R. T. Vanderbilt Company, Inc., 230 Park Avenue, New York 17, N. Y.
Yoder Brothers, Inc., Barberton, Ohio

RELATION OF HYDROGEN-ION CONCENTRATION OF NUTRIENT SOLUTION TO PHYTOPHTHORA ROOT ROT OF AVOCADO SEEDLINGS [1]

Frank T. Bingham and George A. Zentmyer [2]

SUMMARY

Avocado seedlings were grown for several weeks in solution cultures containing nitrogen as either nitrate or ammonium and adjusted to hydrogen-ion concentrations ranging from pH 3.0 to 8.0. *Phytophthora cinnamomi* Rands was introduced into the cultures, and the resulting root-rot development was observed.

Root rot occurred in all inoculated cultures from those of pH 3.5 to those of pH 8.0. There was no disease development at pH 3.0. As the reaction of the solution cultures was lowered from pH 6.0 to 3.5, the rate at which the roots of the host became diseased decreased markedly. Disease development was also somewhat slower at pH 8.0 than at pH 6.0.

The type of nitrogen supplied to the host had no effect on the rate at which root rot developed at a given pH level.

Phytophthora cinnamomi Rands, the causal organism of avocado root rot (3, 9, 10, 11, 13, 16), threatens a considerable portion of the avocado acreage in California unless a suitable means of control is developed. The disease is extensive in occurrence, since instances are reported from such widely distant areas as Puerto Rico (9), Peru (2, 3), Honduras (17), Costa Rica (14), South Africa (10), Mexico (14), and California (11, 13, 16). Furthermore, a number of hosts are identified with the pathogen (8), thus making the problem more serious.

Inasmuch as eradication of an organism of this type and of such wide distribution is out of the question, efforts are being made to circumvent the adverse effects of the organism by research on resistant rootstock, soil amendments, and host nutrition. Several preliminary reports have summarized the status of research on resistant rootstock (15, 18, 19).

Relatively little information has been published on the effect of host nutrition or environmental factors on the development of root rots caused by *Phytophthora cinnamomi*. In a study of rhododendron wilt caused by *P. cinnamomi*, White (12) observed that only 1 plant out of 15 became diseased in naturally infected soil acidified to a pH lower than 4.0. In soil plots adjusted to pH 7.3 or higher, 33 per cent infection occurred, whereas a much greater infection took place when the soil reaction was adjusted to values ranging from 4.0–7.3.

Anderson (1), investigating root rot of pineapple plants grown in nutrient solutions, noted that high salinity and potassium reduced the development of this disease, in which *Phytophthora cinnamomi* is the causal agent. Lewcock (7) concluded that a possible control of Phytophthora root rot of pineapple was offered in acidification to a pH of less than 5.0.

In 1949, Curtis and Zentmyer (4) examined the ease with which avocados growing in nutrient solutions became infected with Phytophthora root rot when various concentrations of oxygen were maintained in the cultures. Root-rot development was observed to be more rapid in the solutions that contained the highest oxygen content. In 1951, Zentmyer (15) and Aldrich observed less Phytophthora root rot with avocados grown for several months in potted soil under alkaline reactions than under acid reactions, but the lowest pH in this experiment was 5.5. Their study consisted of a soil naturally infested with *Phytophthora cinnamomi* and treated in a manner calculated to produce acid to alkaline reactions, with appropriate checks in the same soil steam-sterilized.

To obtain more specific information on the relation of hydrogen-ion concentration to Phytophthora root rot of avocados, the present study was undertaken. A solution culture technique was used, and acidity and nitrogen supply (nitrate or ammonium) were varied in a controlled fashion. This investigation, which was conducted during the spring of 1953 in a greenhouse maintained at a temperature of approximately 70° F., consisted of 2 experiments. In the first experiment cultures containing nitrogen as nitrate were adjusted to different pH values ranging from 3.0–8.0; in the second, 2 separate sets of cultures were adjusted to pH values of 3.0, 3.5, 4.0, 4.25, and 4.50, one set receiving nitrate nitrogen and the other ammonium.

MATERIALS AND METHODS.—Avocado seedlings for these experiments were obtained by germinating Mexican avocado seeds (Topa Topa variety) in sand. Sixty days after planting, the seedlings were transferred to solution cultures containing all the necessary nutrients in sufficient concentrations to provide for the nutrition of the avocado.

A schematic sketch of the nutrient culture apparatus employed is in Fig. 1. The apparatus consisted of a 100-l. glazed ceramic cylinder fitted with a cover and with an air pump to provide proper aeration and mixing of the nutrient solution.

The nutrient solutions of the nitrate series were prepared by adding $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4, respectively, at the rate of 2.5, 1.0, and 1.0 millimoles per liter of culture solution. The ammonium series received, in place of the $Ca(NO_3)_2$, 2.5 millimoles each of $Ca(H_2PO_4)_2$ and $(NH_4)_2SO_4$. With the exception of iron, the minor elements were supplied ac-

[1] Accepted for publication June 10, 1954.
Paper No. 806, University of California Citrus Experiment Station, Riverside, California.

[2] Assistant Chemist, Department of Soils and Plant Nutrition, and Associate Plant Patholiogist, Department of Plant Pathology, respectively, University of California Citrus Experiment Station, Riverside.

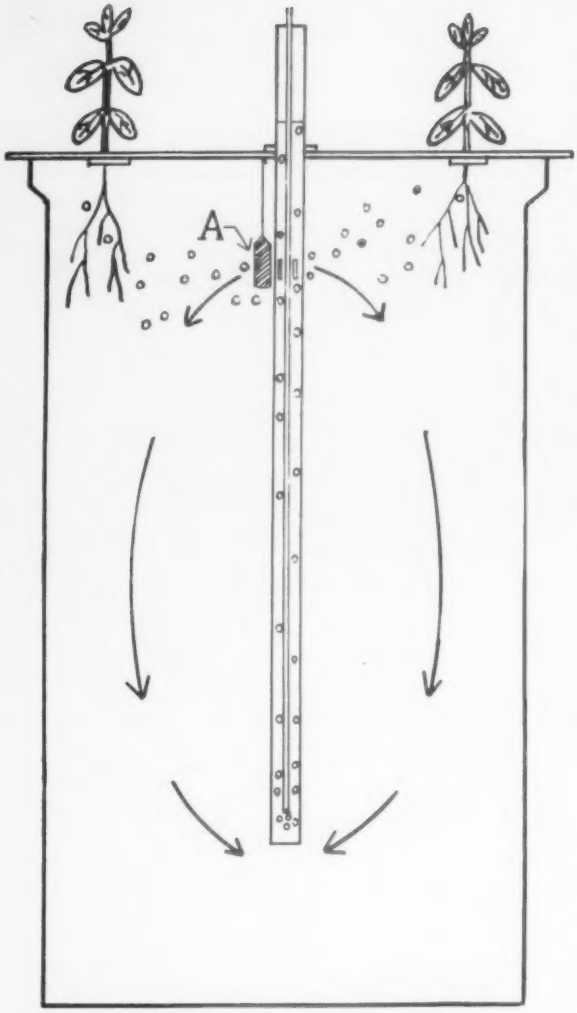


FIG. 1. Schematic sketch of the solution culture apparatus employed in Phytophthora root-rot study. The inoculum bag (A) is suspended in the nutrient culture solution.

cording to the recommendation of Hoagland and Arnon (5) but at ½ the recommended strength, thus producing a solution concentration of 0.25 p.p.m.B, 0.25 p.p.m. Mn, 0.03 p.p.m. Zn, 0.01 p.p.m. Cu, and 0.005 p.p.m. Mo. Iron was added as a salt of ethylene diamine tetra-acetic acid in amounts to produce a solution concentration of 1 p.p.m. Fe. This iron salt was prepared according to the procedure described by Jacobson (6).

Acidities were adjusted to and maintained within 1/10 of a pH unit by using H_2SO_4 or KOH and, in the pH 8.0 cultures, by blending domestic and distilled water.

Packets of sterilized alfalfa stems were inoculated in test tubes with a pure culture of *Phytophthora cinnamomi* Rands. After approximately 2 weeks' growth of the fungus on the stems, the inoculum for the solution cultures was prepared by wrapping packets of the inoculated stems in gauze. The gauze wraps, fashioned into bags, were suspended from lids of the culture solutions in a manner that permitted the inoculum to remain submerged and in the path of the circulating nutrient solution. Examination of Fig. 1 and 2 will reveal additional details.

The avocado seedlings were permitted to develop healthy, bushy root systems in the solution cultures prior to being inoculated with *Phytophthora cinnamomi*. Approximately 1 month was required for good root development. During this interval the pH of each culture was brought to the desired level.

After the introduction of the inoculum, the plants were examined daily in order to note the progress of root rot. The first symptoms observed were brown lesions appearing on rootlets, and when these were noted root samples were taken to the laboratory to be examined and cultured for the presence or absence of *Phytophthora cinnamomi*. After initial appearance of root lesions, wilting of the shoot became evident within a few days. By recording the time required for lesions and subsequent wilting to become apparent, the effect of solution reaction on Phytophthora root rot could be noted in a semiquantitative manner.

RESULTS AND DISCUSSION.—*Experiment 1.*—In this experiment pH levels of 3.0, 4.5, 6.0, and 8.0 were used, and nitrogen was supplied as nitrate. Within 48 hours after inoculation, plants in the pH 6.0 cultures developed root lesions and a slight wilt. Twenty-four hours later the plants had completely succumbed to root rot; the entire root systems were dark brown and rotting, and the shoots were completely wilted. At this time, 3 days after inoculation, plants in the pH 4.5 and 8.0 cultures had only a few lesions and no wilt, while those in the pH 3.0 cultures showed no sign of root rot. *Phytophthora cinnamomi* was recovered from lesions on roots from the inoculated plants in solutions maintained at pH 4.5, 6.0, and 8.0. Five days after inoculation, all plants except those growing in the pH 3.0 solutions were severely wilted and had rotting roots. The described symptoms are illustrated

FIG. 2. View of tilted nutrient culture lids showing position of inoculum bag, aerator, and plants. These are avocado seedlings in pH 3.0 cultures several weeks after the culture at the left had been inoculated with *Phytophthora cinnamomi*. No adverse effects are visible.

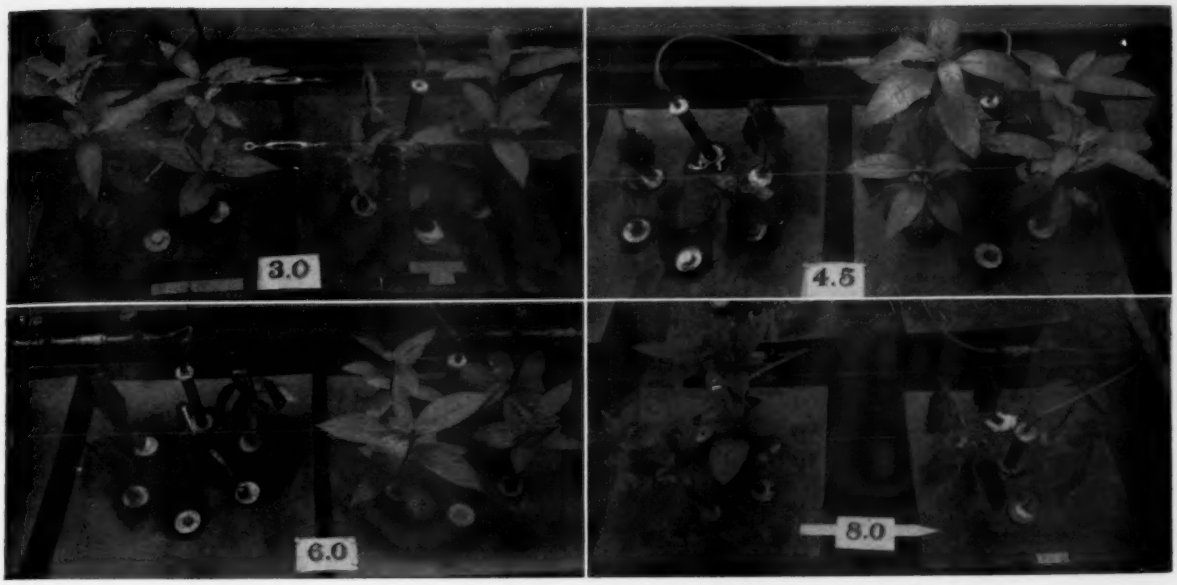


FIG. 3. Effect of solution pH on development of Phytophthora root rot of avocado seedlings. Both of the cultures shown at pH 3.0 were inoculated several times without producing a diseased condition. At pH 4.5, pH 6.0, and pH 8.0 a noninoculated check is compared with a diseased culture.

in Fig. 3 and 4. For comparison purposes, roots of a healthy normal plant and of a diseased plant are presented in Fig. 4.

Plants in the pH 3.0 cultures were inoculated twice more at intervals of about 10 days, but were not affected by the fungus (Fig. 2 and 3). Failure of inoculation at this pH indicates that this hydrogen-ion concentration is higher than the *Phytophora cinnamomi* can tolerate. Since root rot occurred in the pH 4.5 cultures, a critical hydrogen-ion concentration exists somewhere between pH 3.0 and 4.5.

Because of the possibility that the pH 3.0 reaction might have solubilized some trace metal from the walls of the experimental container to the extent that it became fungicidal, A. P. Vanselow, of this laboratory, assisted in evaluating this possibility by making spectrographic analyses of samples of solutions withdrawn from the pH 3.0 and pH 4.5 cultures. No material difference was found to exist between composition of the 2 solutions; therefore, there is no apparent basis for any fungicidal effect of trace metals. Failure of the fungus to cause root rot in pH 3.0 solutions appears to be the result of the hydrogen-ion concentration.

Experiment 2.—The lower limit of acidity at which root rot would still occur, and the effect of nitrate versus ammonium nutrition were studied in Experiment 2. Solution reactions of 3.0, 3.5, 4.0, 4.25, and 4.5 were prepared in 2 sets of cultures which differed only in the type of nitrogen supplied to the plant, i.e., nitrate or ammonium. Typical root-rot symptoms (lesions on roots and wilting of tops) appeared on plants at all pH levels except pH 3.0.

Regardless of pH, there was no difference in the progress of Phytophthora root rot that could be ascribed to the nitrogen source supplied to the host.

The effects of hydrogen-ion concentration of solution on root rot observed in both experiments are summarized in Table 1. Since the type of nitrogen supplied to the plant had no apparent effect on the progress of Phytophthora root rot, data of the nitrate and ammonium series have been combined in this table. Under the conditions imposed by these experiments a critical pH between 3.0 and 3.5 exists for *Phytophthora cinnamomi.* The alkaline limit is apparently somewhat greater than pH 8.0.

These observations are in accord with White's results (12), especially with respect to the extreme acidity required to retard the activity of the fungus. White obtained good control of the organism only when the soil reaction was lowered to pH 4.0 or less, and only partial control when the soil reaction was increased to a pH value greater than 7.3. Zentmyer (15) and Aldrich, in a greenhouse study of the effect of soil reaction upon the incidence of Phytophthora root rot of avocados, obtained some control of disease

TABLE 1.—*Effect of hydrogen-ion concentration on progress of Phytophthora root rot of avocado seedlings in nutrient solution*

Solution pH	Days after inoculation		
	First root lesion	First wilt	Collapse
3.0	—[a]	—	—
3.5	10	15	21
4.0	5	7	10
4.25	3–4	5	7–10
4.50	3–4	5	7–10
6.0	2	3	3–4
8.0	3–4	5	5–6

[a] Dash indicates that plants were resistant.

FIG. 4. Avocado seedlings showing effect of Phytophthora root rot. Left, a healthy plant; right, a diseased plant.

only when the soil pH was made greater than 8.0. The pH of the acid-treated soils was never lower than pH 5.5, however, and this, according to the results of the present investigation and of White (12), is probably not low enough to have any adverse effect on the fungus. Further evidence for having the soil pH lower than 5.5 can be found in the work of Lewcock (7), who recommended lowering the pH of the soil to a value less than 5.0 for the control of pineapple root rot, a disease caused by the same organism.

On the basis of the foregoing evidence, an acid soil pH as low as 4.0 would probably be required for control of *Phytophthora cinnamomi* on avocado. Growth of the avocado seedlings in the acid solutions indicates that the host can grow reasonably well in relatively acid media. Even in the most acid solutions adjusted to pH 3.5 and 4.0, their growth was excellent. However, additional pH studies in the field and in the greenhouse will be necessary for a thorough evaluation of the merits of soil reaction in controlling Phytophthora root rot as well as influencing the nutrition of the host. The matter of low base saturation (low calcium and magnesium, primarily) resulting from soil acidification is a matter that may relate to the nutrition of the host or to invasion of the host by Phytophthora root rot, and this, also, should be studied.

The need for pH control in Phytophthora root-rot investigations is evident from these studies.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. ANDERSON, E. J. 1951. Effect of nutrient salt concentrations on infection of pineapple roots by Phytophthora cinnamomi. (Abs.) Phytopathology **41**: 2.
2. CRANDALL, B. S. 1945. Three phytophthora diseases in the region of Tingo Mario, Peru. U. S. Dept. Agr. Pl. Dis. Rptr. **29**: 536.
3. CRANDALL, B. S. 1948. Phytophthora cinnamomi root rot of avocados under tropical conditions. Phytopathology **38**: 123-130.
4. CURTIS, D. S., AND G. A. ZENTMYER. 1949. Effect of oxygen supply on Phytophthora root rot of avocado in nutrient solution. Amer. Jour. Bot. **36**: 471-474.
5. HOAGLAND, D. R., AND D. I. ARNON. 1950. The water-culture method for growing plants without soil. Cal. Agr. Exp. Sta. Cir. 347. [Revised by D. I. Arnon.]
6. JACOBSON, L. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene-diamine tetra-acetate. Pl. Physiol. **26**: 411-413.
7. LEWCOCK, H. K. 1935. Pineapple wilt disease and its control. Queensland Agr. Jour. **43**: 9-17.
8. THORN, W. A., AND G. A. ZENTMYER. 1954. Hosts of Phytophthora cinnamomi Rands. U. S. Dept. Agr. Pl. Dis. Rptr. **38**: 47-52.
9. TUCKER, C. M. 1928. Report of the Plant Pathologist. Avocado root disease. Puerto Rico Agr. Exp. Sta. Rept. **1928**: 29-32.
10. WAGER, V. A. 1941. The dying-back or decline disease of avocado trees. Farming in South Africa **16**: 185-186.
11. WAGER, V. A. 1942. Phytophthora cinnamomi and wet soil in relation to the dying-back of avocado trees. Hilgardia **14**: 517-532.
12. WHITE, R. P. 1937. Rhododendron wilt and root rot. N. J. Agr. Exp. Sta. Bul. **615**: 1-32.
13. ZENTMYER, G. A. 1946. Diseases of the avocado. Cal. Avocado Soc. Yearbook **1946**: 79-83.
14. ZENTMYER, G. A. 1952. Phytophthora cinnamomi on avocado in Mexico and Costa Rica, and other avocado diseases in Mexico. U. S. Dept. Agr. Pl. Dis. Rptr. **36**: 31.
15. ZENTMYER, G. A. 1952. Research on avocado root rot. Cal. Avocado Soc. Yearbook **1952**: 103-106.
16. ZENTMYER, G. A., AND L. J. KLOTZ. 1947. Phytophthora cinnamomi in relation to avocado decline. (Abs.) Phytopathology **37**: 25.
17. ZENTMYER, G. A., AND W. POPENOE. 1951. Phytophthora cinnamomi on avocado in Honduras. U. S. Dept. Agr. Pl. Dis. Rptr. **35**: 25.
18. ZENTMYER, G. A., AND C. A. SCHROEDER. 1951. Resistance of subtropical plants to Phytophthora cinnamomi. Cal. Avocado Soc. Yearbook **1951**: 105-106.
19. ZENTMYER, G. A., AND C. A. SCHROEDER. 1951. Research on rootstocks resistant to avocado root rot. Cal. Avocado Soc. Yearbook **1951**: 157-158.

EFFECT OF TEMPERATURE AND HUMIDITY ON THE GRAPE POWDERY MILDEW FUNGUS[1]

Charles J. Delp[2]

SUMMARY

A method was devised for mounting epiphytic fungi in celloidin films for study, and a progression of *Uncinula necator* (Schw.) Burr. development was illustrated. Conidial germination of grape powdery mildew was studied on dry slides and on host leaves at different temperatures and was found to be essentially the same at comparable temperatures. The minimum temperature for germination was below 6°C.; for infection and growth it was about 7°C. Rapid germination, infection, and growth took place from 21° to 30°C., and 25°C. was the optimum for germination. Infection did not occur at a leaf temperature above 31.5°C. and germination ceased about 33.5°C. At about 31°C. the conidia produced were less than 10 per cent viable. Exposures of about 10 hours above 34°C. or 4 hours at 40°C. killed conidia in mildew colonies and on glass slides. Vigorously growing mildew colonies were killed on leaves heated to about 34°C. for 2.5 days, 36°C. for 10 hours or 39°C. for 6 hours.

VPD is the best indication of moisture stress but neither RH nor VPD should be used independently of temperature when discussing the influence of humidity on powdery mildew. Germination of conidia on dry slides was not influenced by low humidity at temperatures up to 25°C. At 28°C. a slight reduction in germination occurred above 21 mm. VPD. Germination occurred in dry air at 33°C. and 38 mm. VPD; at 29°C. and above there was a significant reduction in germination above 8 mm. VPD. There was a reduction in germination at all temperatures when exposed to a humid atmosphere of 0 mm. VPD, and water condensed on the slide. On grape leaves, spore germination, infection, and growth occurred in a normal manner under all atmospheric moisture stresses from 25–31°C. These humidity tests were made in a regulated air stream of constant humidity and temperature passed over attached leaves in a tunnel described and pictured.

Other environmental factors being comparable, the host plant did not appreciably influence spore germination. However, infection, growth, and sporulation were in an inverse proportion to the maturity of the host leaves, and berries of several varieties resisted infection by powdery mildew after they matured to 8 per cent sugar content or above.

Among conidia remaining in chains, germination started generally from one end. Terminal and internal conidia of a chain germinated equally, but the total germination decreased as the chain length increased. Germination of spores *in situ* was not observed.

The results of these tests were used to explain the occurrence and natural control of grape powdery mildew in areas of California.

During some seasons powdery mildew of grape, incited by *Uncinula necator* (Schw.) Burr., is difficult to control even in the interior valleys of California. This may be due to neglect of proper control procedures or to the occurrence of conditions exceptionally favorable for development of the disease. If the role of temperature, moisture, light, and the variety, culture and maturity of the host plant in development of the fungus were known, more effective control practices could undoubtedly be formulated. A study was made of the influence of temperature, humidity, and some factors of host maturity on the grape powdery mildew fungus.

CELLOIDIN STRIPPINGS TO STUDY FUNGUS DEVELOPMENT.—A modification of Wenzil's celloidin membrane method (32) was used to study germination, infection, and growth of the powdery mildew fungus on grape leaves and fruit. Commercial celloidin was spread thinly over the plant surface with a glass rod and allowed to solidify for about a minute. After the solution had solidified, but before it had begun to shrink, the film was stripped from the plant surface, stained in 10 per cent cotton blue in lactophenol and mounted on a slide. Care was taken to avoid excessive drying, which caused the film to become translucent and shrivelled. All fungus structures outside the cuticle were removed with the stripping, which also contained plant hairs and an impression of the epidermal cells.

CONTROLLED-ATMOSPHERE WIND TUNNEL.—A controlled-atmosphere wind tunnel (Fig. 1) was constructed for studying the relation of humidity and temperature to mildew development on single attached leaves. Tunnel I accepted a given atmosphere, allowed the air to homogenize, and directed a streamlined air current into tunnel II which contained the attached leaf under study. A trap door in the bottom of tunnel II allowed for the insertion of the leaf which was held in place on a wire rack by small bits of modeling clay. The top was covered by a glass plate and illuminated by 200 foot candles from blue fluorescent lights. Mounted on this glass plate, and passing through a ¼ in. hole in it, was a rack which supported a thermocouple. The rack was made of a glass tube attached to a microscope mechanical stage for raising and lowering the thermocouple over the leaf. The thermocouple was made of 40 gage (diameter 70μ) copper and constantan wire according to the directions by Lorenzen (21). A nylon wick was arranged to hold a

[1] Accepted for publication June 23, 1954.

Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California.

[2] Formerly Graduate Assistant, Department of Plant Pathology, University of California, now Senior Research Investigator, E. I. du Pont de Nemours and Company.

The author acknowledges the guidance of Dr. Wm. B. Hewitt, Professor, Department of Plant Pathology, University of California, under whose supervision the work was conducted.

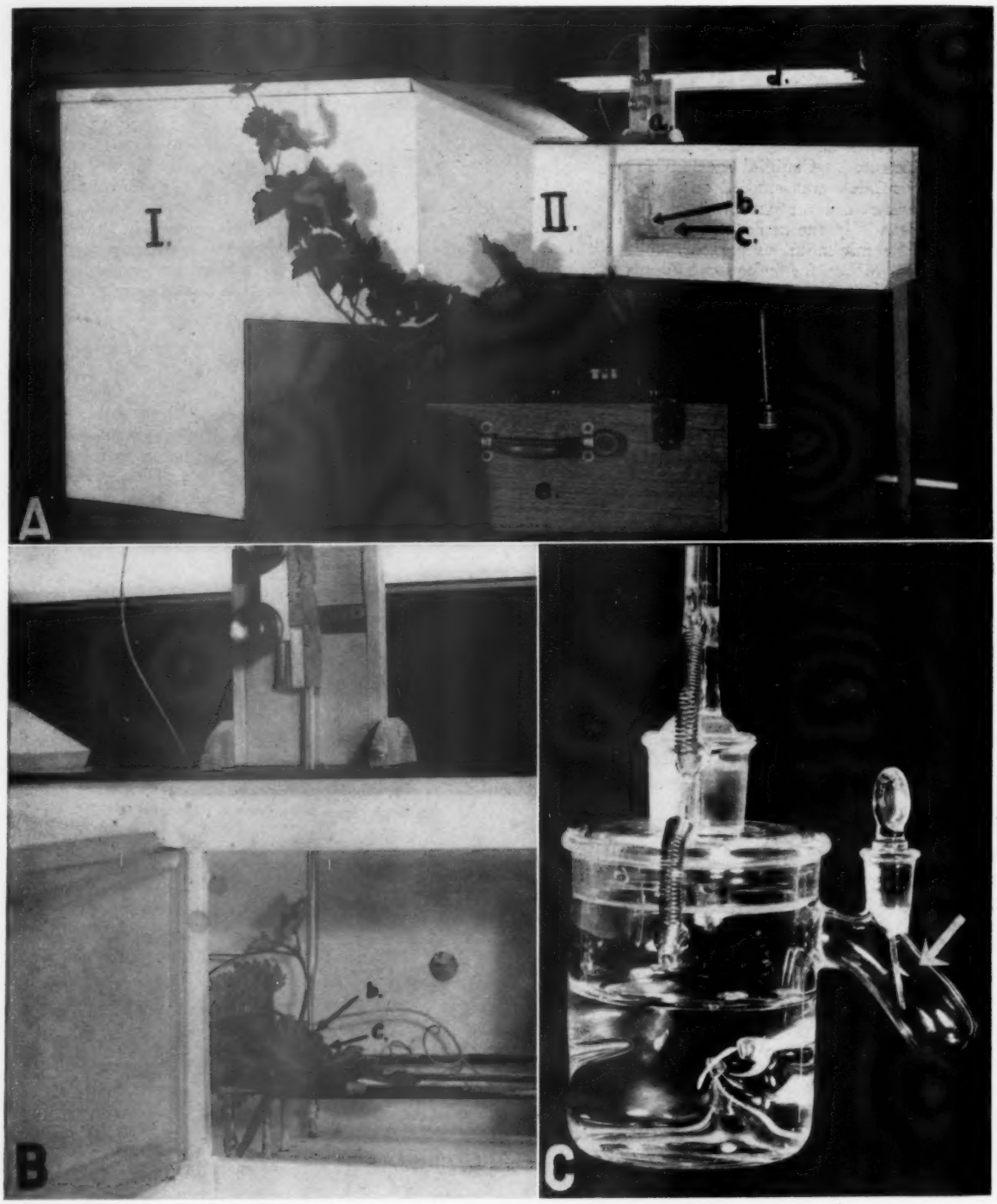


FIG. 1. Equipment used in controlled humidity studies of powdery mildew of grape: A) Overall view of the wind tunnel used to pass a regulated air stream over attached leaves; I is the receiving and homogenizing section and II is the air tunnel containing the leaf under study. Adjustable rack (a) supports a small thermocouple (b) over the test leaf (c) illuminated by a fluorescent light (d). Potentiometer (e) is used in temperature and humidity readings. B) A portion of tunnel II through the opened side door showing the position of the thermocouple (b) and a leaf (c). C) A 150 ml. Warburg apparatus flask containing 75 ml. of humidity-regulating solution and a glass slide (arrow), covered with powdery mildew conidia, inserted in the side-arm.

drop of distilled water for wet bulb humidity readings. Millivolt readings were taken with a Leeds and Northrup 8662 potentiometer, and an ice pot was used for a constant reference temperature. A plastic door in the side of tunnel II was removable to allow for internal adjustments.

Two different sources of regulated atmosphere were used. When humidities and temperatures above those of the laboratory were required, the air was tapped from a constant temperature recirculating experimental dehydrator designed and maintained by the Agricultural Engineering Department of the University of California at Davis. In this chamber the humidity was regulated by steam injection. When required, the humidity was controlled to ± 0.5 mm. VPD by passing the air through 2 36-cu. ft. refrigerators. The dew point was regulated by the temperature of the refrigerators. This air was then taken into a 12 cu. ft. chamber where it was heated to the desired temperature and blown into the tunnel. The controlled atmosphere passed over the leaves at about 1 mile per hour.

Humidity will be expressed in this paper as per cent relative humidity (RH) and, as the more precise measure of moisture stress, mm. of vapor pressure deficit (VPD). The use of VPD and a concept of atmospheric humidity are reviewed by Anderson (1) and Stevens (28). The VPD can be determined from psychrometric readings. It is the reciprocal of the RH percentage of the vapor pressure of water at any given temperature. With a change in temperature, the RH remaining constant, the moisture stress or VPD changes.

INFLUENCE OF TEMPERATURE ON GRAPE POWDERY MILDEW.—One of the first reports of temperature influencing grape powdery mildew was a statement in the Gardener's Chronicle by Lindley in 1852 (19). "I recollect some time since being told by a person who stated that he had several large vineries under his superintendence, that he arrested the mildew entirely by creating a high temperature, without the use of sulphur or anything else, which, if correct, is worth knowing, but not having tested the advice, I cannot speak to its accuracy." There have been a number of field observations reporting the following cardinal temperatures for mildew development: minimum temperatures 6°C. (7), 10°C. (3, 18) and 12°C. (22, 31); optimum temperatures 20–25°C. (7), 25–35°C. (22, 31), 29–35°C. (30), and 32–35°C. (3, 18); and maximum temperatures 38°C. (3, 18, 30) and 40°C. (22, 31). Yossifovitch (39) concluded that the minimum, optimum, and maximum temperatures for spore germination were 4.5°–6.5°C., 25°–28°C., and near 35°C., respectively. Yarwood et al. (38) reported slight hyphal growth at 0°C., maximum growth at about 26°C., and no growth at 36°C.

Relation of temperature to spore germination.—A study was made of the germination of conidia on dry glass slides at different temperatures. A spore source for all experiments was maintained on potted grape plants grown under 12 hours of artificial light in the laboratory, since it was found that sporulation was abundant on plants kept at moderate temperatures under diffuse light.

Uniform distribution of conidia on slides was attained by gently blowing conidia from the leaves onto slides placed in the bottom of a 30-gal. can. At the conclusion of germination tests, conidia were killed and stained in 5 per cent cotton blue in lactophenol. This procedure allowed accurate time intervals for experiments, assured no additional germination after removal from experimental conditions, and aided in germination counts.

Conidia produced in the laboratory nursery were dusted onto dry slides, suspended over water in moist chambers, and then incubated at different temperatures for 24 hours. The complete series in 3°C. intervals was repeated 4 times. Two additional tests were made at 1°C. intervals between 29° and 33°C., and 3 tests at 1°C. intervals between 24° and 27°C. Data from these experiments were combined with data from the Warburg humidity tests and tests on dry slides in the wind tunnel, to be discussed later. To facilitate the combination, these data were figured as a percentage of a check for each spore lot. The check was determined by the highest germination obtained in a humid atmosphere (1-3 mm. VPD) at 24° and 27°C. Check germination in different tests varied from 40 to 80 per cent. The germination percentage for each temperature in the following tabulation is an average of at least 1000 spores.

Temperature (°C.)	6	12	15	18	21	24	25	26	27	28	29	30	31	32	33	34
Germination %	3	6	9	37	80	95	100	96	90	78	64	53	40	31	3	0

Seldom were conidia found to be germinated immediately after removal from host plants—in no case did such germination exceed 0.5 per cent. This verified the observation that germination did not take place *in situ*.

Studies of germination on attached leaves were made in plant growth chambers and in the controlled-atmosphere wind tunnel. Celloidin strippings were used to obtain exact counts of germination. Leaf and air temperatures were measured with the small thermocouple. The germination of conidia on glass slides in the chambers with the plants was compared with germination on the host. At each temperature, germination on leaves was approximately the same as on dry slides except at 32° and 32.5°C.; on leaves at 32-32.5°C. spore germinations as high as 50 per cent were obtained in 10 separate tests, whereas in only 1 case did germination exceed 30 per cent on dry slides at 32°C. An explanation for this difference was not evident. No germination occurred at 34°C. leaf temperature.

In tests on the rate of spore germination at different temperatures, conidia were dusted onto coverslips which were placed in petri dishes in incubators regulated at different temperatures. At prescribed times, 1 coverslip was removed from each incubator

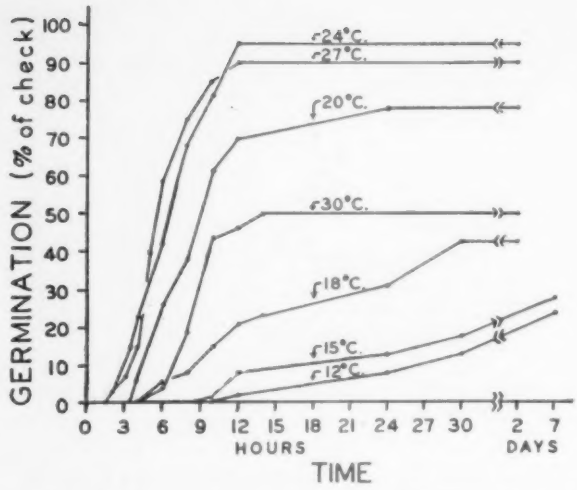


FIG. 2. The percentage of germination of grape powdery mildew conidia at different temperatures after different intervals of time. The optimum for germination is between 24° and 27°C. Each point represents an average of 600 spores.

and inverted in a drop of cotton blue in lactophenol. Two hundred spores were counted on each coverslip to determine the percentage of germination. This test was repeated 3 times, and the replicates were combined (Fig. 2) by figuring the germination as a percentage of the check, which was the highest germination for each replicate (40, 42 and 50 per cent). At 24° and 27°C., germination had begun after 1.5 hours and had reached a maximum at 12 hours. No indication of germination was evident at 18°, 20°, and 30°C. prior to 3.5–4 hours. Germination reached a maximum at 30°C. after 14 hours, at 20°C. after 14–24 hours, and 18°C. after 24–30 hours. The first indication of germination at 12° and 15°C. was evident after 8 hours, and germination continued very slowly at these temperatures.

Appressoria were formed on almost all germ tubes which came into contact with a solid surface (Fig. 3c). The initial swelling of germ tubes occurred within 2 hours after the germ tubes began to form, and the complete appressorium had developed within another 2 hours.

Influence of temperature on infection, growth, and sporulation. — Fungus development was studied in plant-growth chambers with artifical light. Below 10°C. constant-temperature refrigerator rooms with temperature control of ±0.5°C. were used. Between 15° and 30°C. chambers similar to those described by Hartmann and McKinnon (14) were used with a temperature control of ±1°C. Above 30°C., a smaller and modified form of these chambers was used with a temperature control of ±0.5°C. In all these chambers air was circulated at a rate of 1–3 miles per hour.

The *Vitis vinifera* plants used in all experiments were potted rootings of the Carignane and Emperor varieties, and seedlings from open-pollinated Emperor vines—all highly susceptible to powdery mildew. To avoid the use of fungicides in the control of powdery mildew, experimental plants were kept in a separate greenhouse, and the leaves were drenched daily with water. This procedure gave adequate control. All tests were conducted on leaves 1–4 weeks old because of the reduced development of powdery mildew on older leaves.

Potted grape plants were inoculated and placed in plant-growth chambers at different temperatures. Periodically, celloidin strippings were taken to observe the development of the mildew colonies. Normal germination (Fig. 3c) resulted in the formation of a single germ tube terminated with an appressorium. This, unlike that reported for *Erysiphe graminis* (8), was normal behavior at all temperatures and took place on any solid surface. On host tissue, primary infection from the germ tube appressorium was first indicated by the formation of a primary hypha (epiphytic hypha produced from the conidium subsequent to primary infection) from a location on the conidium opposite the germ tube. A secondary and, generally, a tertiary hypha were produced from the original appressorium or from the conidium near the germ tube. Mycelium from these hyphae branched and radiated to form a more or less circular colony, and eventually conidiophores and chains of conidia were produced. The percentage of germination and infection was determined, and growth was observed until the formation of conidiophores and primary conidia was evident. Two to 5 replications were made at each temperature. Leaf temperatures, as measured with the small thermocouple, were used to interpret the results, since the air temperature was 0–3°C. above or below the leaf temperature.

The results from these tests (Table 1) show that the minimum leaf temperature for spore germination was below 6°C., but infection did not take place below 7°C. Growth was very slow at 7°C. with more than 32 days from inoculation to sporulation. At 9°C. this period was reduced to 25 days. The growth rate increased with a rise in temperature to a maximum at about 26°C. The time from inoculation to sporulation was 5 days at 26°C. and 6 days at 23° and 30°C. As previously stated, germination occurred up to 33°C., but no infection took place above 32°C, at both high and low humidities (2 and 32 mm. VPD). The grape plants did not continue to grow in the chambers below 10°C.

In another experiment young excised leaves were collected from the field and greenhouse, washed and floated on tap water. These were inoculated and placed in incubators at a series of temperatures. Celloidin strippings were taken daily for 7 days to study germination, infection, and development. This test was replicated 5 times. The most rapid development occurred at 27°C. followed very closely by colony development at 30°C. At these air temperatures, 6 days elapsed from inoculation to primary spore production. Colonies grown on excised leaves in the 24°C. incubator had profuse branching growth but no spore production in 7 days. Germination of 40 per cent of

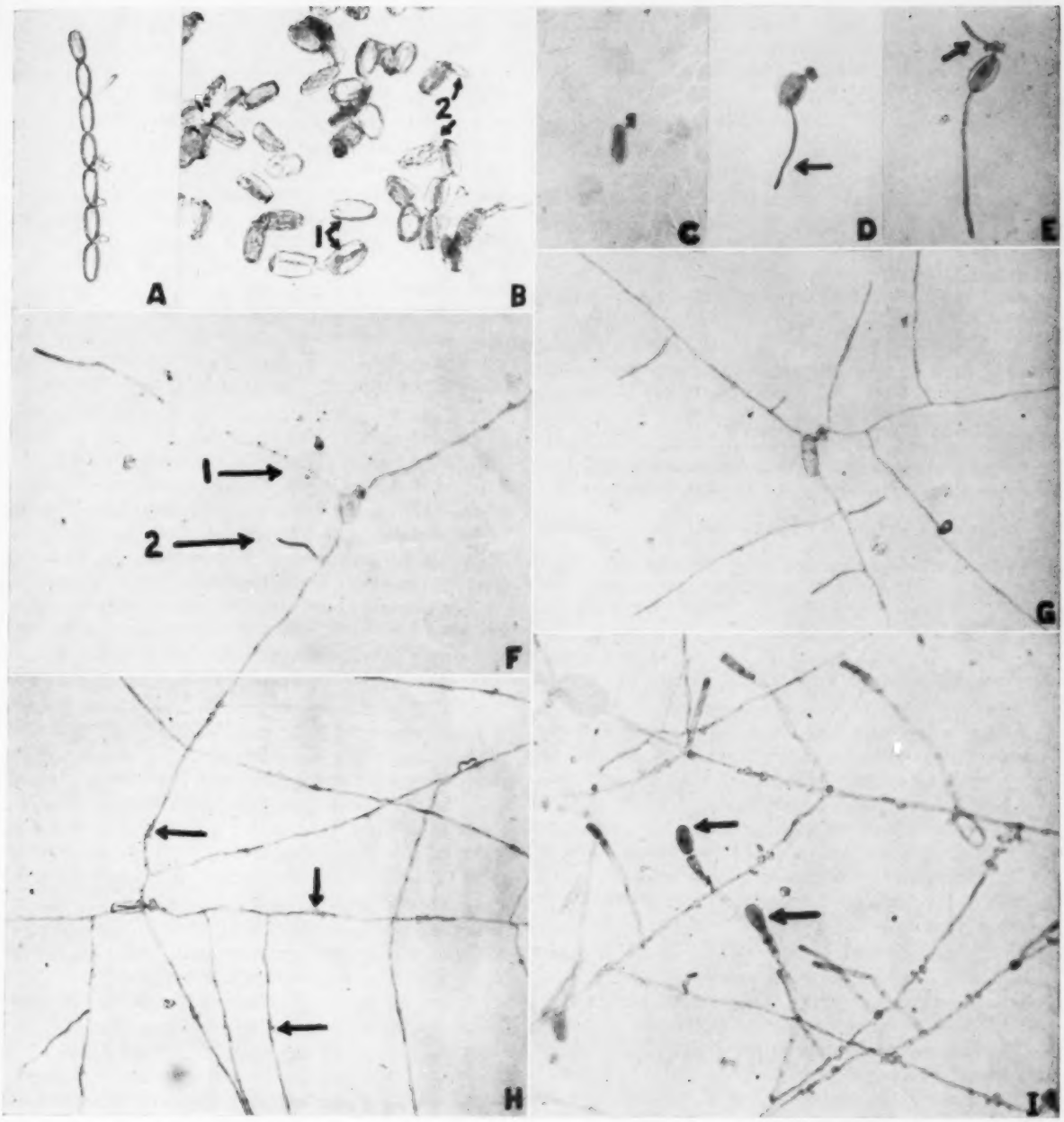


FIG. 3. The typical progression of development of grape powdery mildew from germination to sporulation. Magnification is approximately 100 ×. A and B from glass slides, C–I from celloidin strippings stained in cotton blue. A) Germination of conidia attached in a chain. The bottom 4 conidia have formed normal germ tubes terminated by appressoria. The germ tube of the fifth spore grew into the air, and, as was generally the case with germ tubes that failed to contact a solid surface, lacked an appressorium. B) Abnormal germination in water. Note the ruptured or plasmoptized (1) and non-germinated conidia and germination by the production of a short germ tube lacking an appressorium (2). C) Normal germination with a short germ tube terminated in an appressorium. Notice the outline of epidermal cells in the background. D) Infection, first indicated by the formation of a primary hypha (arrow) from the end opposite the germ tube. In these studies the hypha and the following stages of development were observed only on susceptible host tissues. E) Secondary hypha (arrow) arising from the primary appressorium. F) Tertiary hypha (1) arising from the germ tube end of the spore and the first indication of branching (2) on the primary hypha. G and H) Continued growth and branching. Notice the appressoria on the mycelium (arrow). I) Primary conidia (arrow) forming on conidiophores.

TABLE 1.—*Development of grape powdery mildew on grape plants subjected to different temperatures in plant growth chambers*

Leaf temperature °C.	Chamber temperature °C.	Germination %	Infection[a] %	Time from inoculation to sporulation (days)
6 ± 1.0	5 ± 0.5	45	0	—
7 ± 1.0	6 ± 0.5	20	1–5	32
9 ± 1.0	8 ± 0.5	20	1–5	25
12 ± 1.0	10 ± 0.5	50	1–5	18
11 ± 3.0	10 ± 2.0	45	15	16
12 ± 1.5	11 ± 1.0	55	20	12
15 ± 1.5	14 ± 1.0	25	20	11
17 ± 1.5	16 ± 1.0	50	20	7
23 ± 1.0	23 ± 1.0[c]	60	25	6
26 ± 1.0	27 ± 1.0	70	40	5
30 ± 1.0	32 ± 1.0	50	15	6
31 ± 0.5	33 ± 0.5	40	0–15[b]	—
32 ± 0.5	34.5 ± 0.5	20	0	—
32.5 ± 0.5	33 ± 0.5[c]	15	0	—
33.5 ± 0.5	34 ± 0.5[c]	0	0	—
33.5 ± 0.5	35 ± 0.5	0	0	—

[a] Infection is indicated by subsequent growth, the production of a primary hypha (Fig. 3), after an appressorium is formed.

[b] In 1 test 15 per cent infection occurred, but in a replication no infection took place.

[c] Transpiration was low due to the high humidity (<1 mm. VPD. Thus the leaf temperature more nearly approached the air temperature. In the other tests the humidity was moderate (<15 mm. VPD).

the conidia occurred in the 33°C. incubator, but no infection took place.

Lethal effect of high temperature.—Sporulating powdery mildew colonies and detached conidia on dry glass slides were subjected to high temperatures for different periods of time. The effect of these treatments on spore viability was determined after exposure by testing the germination of the conidia on slides at 24°C. Sporulating colonies were produced on potted plants in the laboratory nursery. The germination of conidia from these colonies was 40–60 per cent.

In a chamber held at 32° ±1°C. (21 and 24 mm. VPD) the leaf temperature averaged about 1.5°C. below the air temperature. Good spore germination, infection, growth, and sporulation of the powdery mildew colonies took place in the chamber. The germination of conidia produced was low and never greater than 10 per cent. After 24 hours' exposure in the 32°C. chambers, less than 10 per cent of the conidia in colonies on plants was found viable.

Neither germination of spores nor further growth of established colonies occurred on leaves with temperatures which ranged between 33° and 35°C. in a chamber regulated at 35° ±1°C. The germination of conidia from colonies on leaves subjected to this temperature dropped to 10 per cent and less in 5–10 hours and to less than 1 per cent in 10–20 hours.

Conidia dusted on coverslips and placed in petri dishes, both dry and suspended over water, were placed in incubators regulated at 35° and 36°C. Periodically a coverslip containing conidia was removed and incubated at 24°C. to determine viability. In all tests germination dropped to 0 within 10–12 hours. In a 40°C. incubator conidia were dead after a 4-hour exposure.

To determine the time of exposure at high temperatures required to kill powdery mildew colonies, plants with vigorously growing mildew colonies were exposed in plant growth chambers regulated at 35.5°, 37.5°, and 40.5° ± 0.5°C. Leaf temperatures were about 2.5°C. below the chamber temperatures. Upon removal, tests were made on spore viability and each plant was immediately covered with a cheesecloth cage or plastic bags to prevent contamination. These tests were repeated at 5 and also at 10 days after removal from the temperature chambers to determine whether death or recovery had occurred. Plants were kept in diffuse light and moderate temperature in the laboratory during the 10-day observational period.

In these tests, less than 10 per cent of the conidia germinated and no infection was observed on inoculated plants exposed in a chamber regulated to 35.5°C. Also, conidia which had germinated on leaves of potted plants in the laboratory failed to cause infection when exposed in this chamber. New infections produced in the laboratory were not killed in 24 hours' exposure in this 35°C. chamber. Young sporulating colonies recovered in 5 days from a 2-day exposure, but were completely killed by an exposure of 2.5 days or more. Changing the moisture stress from 15 to 25 mm. VPD had no noticeable influence on these results.

After 10 hours' exposure at 37.5°C. the colonies on 1 plant recovered but on 2 other plants failed to recover. All colonies exposed for 9 hours or less recovered, but exposures longer than 10 hours consistently eradicated the mildew. Daily exposures of 4 hours each for 4 days, and 6 and 8 hours for 2 days, failed to kill the colonies. Changing the moisture stress from 14 to 38 mm. VPD caused no significant difference in these results.

On plants exposed for 4 hours at 40.5°C. the powdery mildew colonies recovered only slightly in 5 days. They had almost completely recovered in 10 days. Exposure for 6 or more hours in the 40.5°C. chamber killed the fungus.

Mildew colonies, which had been sporulating abundantly and were a white mass of mycelium and conidia, often retained their original microscopic appearance after having been killed by high temperatures. They could be distinguished from viable colonies by microscopic examination to reveal shrivelled conidia and by testing the conidia for viability. The host often exhibited a necrotic flecking or a complete death of the infected epidermal cells beneath colonies of all ages when subjected to high temperatures. This reaction took place under various conditions which failed to kill the colony as was described in 1853 by von Mohl (23). Under conditions where most of the conidia were removed, generally by air movement, the colony appeared as a shiny chlorotic area. Powdery mildew colonies and infected host tissue which resembled

those killed in the laboratory by heat treatments have frequently been observed in the vineyards of central California. Temperatures as high as 40°C. for 1-day periods had no noticeable deleterious effect on the healthy portions of the grape plants used in these experiments.

INFLUENCE OF HUMIDITY ON GRAPE POWDERY MILDEW.—Field observations have led to the supposition that free water may be necessary for grape powdery mildew development (12) and that rain and fogs are very favorable (3). On the other hand precipitation has been observed to have an adverse effect on powdery mildew (30). The high humidities attained during dews (30) and dull cloudy periods (11), in low places near river banks (18), and in weedy or irrigated vineyards with poor air drainage (11,12) are reported to be most favorable to powdery mildew. It has been stated that fungus development is stopped and spores will not germinate in a dry atmosphere (31,39), and bright sunny days with crisp, dry air are altogether unsuitable for powdery mildew (11). Others consider mildew more severe during dry seasons (24) or dry weather alternating with slight rain (7). Uppal (30) observed that the fungus developed over a range of atmospheric humidity (RH from 40–95 per cent). Yossifovitch (39) in laboratory experiments obtained good conidial germination on dry slides between 20 and 30 per cent RH at 21–33°C. but no germination over sulfuric acid. He found that when inoculated plants were kept in a dry, well-ventilated greenhouse, mildew developed more slowly than under more humid conditions.

The powdery mildews have been classified as dry-weather fungi (34), and some will tolerate the most severe moisture stresses (5,34). The development of others is limited by low humidities (2,20). It cannot be determined from existing information into which group grape powdery mildew falls.

Effect of humidity on spore germination.—Conidial germination on dry glass slides was tested under different moisture stresses at different temperatures. Conidia were dusted onto coverslip strips 4.5 × 22 mm., which were immediately mounted on glass stoppers and inserted through a 5 mm. opening into the side-arm of flasks (Fig. 1c) containing humidity-regulating solutions. This procedure disturbed only a small portion of the equilibrated atmosphere in the side-arm. No spore remained in the laboratory for more than 30 minutes after removal from the host before being exposed to a regulated atmosphere.

A Warburg apparatus was used in the humidity studies as a constant temperature bath control of ±0.1°C. and as a means of agitating humidifying solutions to avoid stratification of the atmosphere and solutions. Manometers were used to determine the time at which a temperature-humidity equilibrium was reached. To assure equilibrium, the flasks (Fig. 1c) containing humidity-regulating solutions were agitated in the constant temperature bath for more than an hour before each experiment.

Humidity-regulating solutions, as described by Stevens (28), Shaw (26), and Wilson (33), modified by Hepburn (17), were prepared with C. P. sulfuric acid, C. P. sucrose, and distilled water. The humidities obtained by these solutions were not measured since these values have been determined by many other workers (17,33).

Conidia on dry glass slides were exposed to the different moisture stresses at a constant temperature for 24 hours. The slides were removed and the spores immediately killed and stained. Check samples were germinated over 2 M sucrose in incubators at 24° and 27°C. to determine the germination potential for each lot of spores at each temperature. This was an aid in comparing tests at different temperatures (Fig. 4), for the germination for each lot varied from 38–65%. The data from these studies are averages of 2 or 3 replications. Each replication represents a count of 1,000 spores per treatment to determine the germination percentage.

Results of these tests (Fig. 4) reveal no significant difference in conidial germination due to moisture stress at or below the optimum temperature represented by 20° and 25°C. At 28°C. there was a significant reduction in spore germination above 21 mm. VPD. As the temperature increased above the optimum for germination to 30°, 31°, and 32°C., there was a slight but significant reduction in germination above 8 mm. VPD. Nine replications were made at 33°C., but germination was too low, due to the high temperature, to determine accurately the influence of low humidities. Some conidia germinated at a moisture stress of 38 mm. VPD. Though the percentage of spore germination was influenced only slightly by exposure to low humidity at high temperatures, the germ tubes were

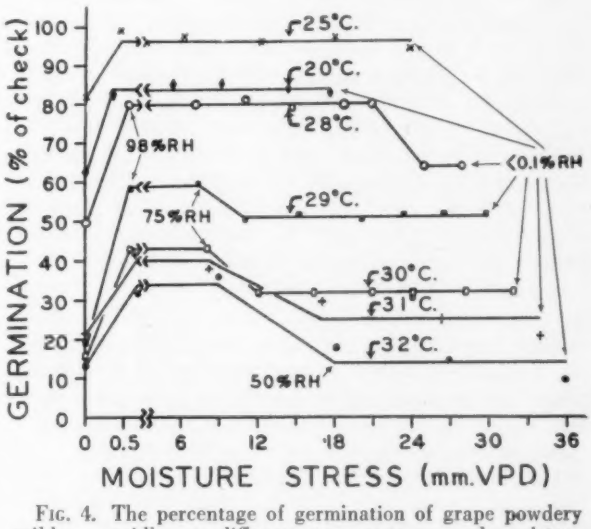


FIG. 4. The percentage of germination of grape powdery mildew conidia at different temperatures and moisture stresses showing the relation of moisture stress (VPD) to temperature and their combined influence on spore germination. The maximum moisture stress (VPD) at 20°C. (0.1% RH) is just half that attainable at 32°C.

frequently shorter with incomplete appressorial formation, and the conidia shrivelled more rapidly than when exposed to less moisture stress. It is evident that the influence of moisture stress on powdery mildew is closely related to temperature. In all tests the conidia on slides suspended over water at 0 mm. VPD germinated poorly and in an abnormal manner (Fig. 3b and 4). Water condensed on these slides, and many workers have noted poor germination of powdery mildew conidia in water (10,15,20).

The germination of conidia on attached leaves under different moisture stresses at different temperatures was tested in the controlled atmosphere wind tunnel previously described. Leaves were inserted into the tunnel and allowed to come to equilibrium for more than an hour. The side door was then removed, and conidia were blown from diseased plants onto the leaf and a glass coverslip mounted in the side of the tunnel. After 12 hours' exposure in the air stream controlled at a constant humidity and temperature, spores were removed from the leaves in celloidin strippings for study. The dry coverslip containing spores was also removed and inverted in lactophenol.

The microclimate in which the spore was germinating was of prime concern in these studies. The influence of temperature was due to the temperature of the leaf surface and not directly to that of the air passing over the leaf. Leaf temperatures, as measured by the small thermocouple placed on the leaf, served as a means of comparing data. The moisture stress was also modified by the transpiring leaf, and in all atmospheres except those of less than 1 mm. VPD, a gradient of wet bulb temperatures could be detected by moving the wet thermocouple down toward the leaf surface. A gradual increase in wet bulb temperature occurred as the thermocouple was lowered from 1.5 mm. to 0.2 mm. above the upper surface of the leaf. Below 0.2 mm. there was a sudden increase in wet bulb temperature. This increase was due to the interference of the moisture of the wet bulb with the moisture gradient, as explained by Ramsay et al (25), and cannot be used to indicate the humidity. The VPD value obtained just prior to the rapid increase was used as the lowest VPD of the gradient. At the higher range of humidity (10–12 mm. VPD) the moisture stress was reduced about 4 mm., but a reduction of about 8 mm. VPD occurred in the dryer atmospheres at temperatures above 30°C. where the atmospheric VPD reached 34 mm.

It was not considered that this method nor any other method found in the literature (25,37) was adequate for a precise measure of the humidity in the microclimate of a germinating spore on a transpiring leaf. For this reason only the moisture stress of the atmosphere which was passed over the leaf is considered. From the author's data, described above, and from information reported by Yarwood (37), Longree (20), Thut (29), and Ramsay et al (25), it is considered that in the low humidity environments the moisture stress at the surface of a transpiring grape leaf is about ¼ less than that of the surrounding air.

In 36 tests in the controlled-atmosphere wind tunnel (Fig. 1) the leaf temperature was varied from 23.5–33.5°C., and the humidity was varied from 1–36 mm. VPD. At a given temperature the germination of conidia on grape leaves and on dry slides was not influenced by changing the moisture stress. In 7 tests each, at 31.5°C. and 32.5°C. (leaf temperature) the variation in germination between high humidity tests was as great as the variation between high and low humidity tests. Atmospheric moisture stresses of 18 mm. VPD at 24°C. (leaf temperature) and 34 mm. VPD at 31°, 31.5° and 32.5°C. caused no significant reduction in germination when compared with germination in high humidities at comparable temperatures. When it is considered that 24 mm. VPD is equal to 0 per cent RH at 25°C., it can be seen that these moisture stresses (up to 36 mm.) afforded a severe test.

Influence of humidity on infection and growth.—Attached grape leaves were inoculated and immediately placed in the controlled-atmosphere wind tunnel where the fungus was subjected to different temperatures and humidities. Periodically celloidin strippings were made to determine the progress of fungus development. It was observed (Table 2) that normal germination, infection, and growth to the stage of conidial production took place in 5 days at a VPD of 25 mm. at 27°C. (leaf temperature), and infection occurred at leaf temperatures as high as 31°C. under both humid and dry conditions (atmospheric moisture stresses of 1 and 34 mm. VPD). In the low humidity studies it was noted that germination (about 40 per cent took place on leaves up to 33°C., but infection did not occur above 31.5°C. This difference in maximum temperature between infection and germination was thought to have been due to a desiccation of the germ tubes before infection could be established. In tests made under moisture conditions where the conidia could not dry out (1 mm. VPD) the response was generally the same; germination but no infection occurred at 32.5°C. This indicated that the response was due to temperature alone and not to a low humidity.

The higher temperature range (29–33°C.) was more thoroughly tested in these experiments since only at the highest temperature can the maximum moisture stress be obtained. At 32°C. (Table 2) when germination but no infection took place, the conidia were dead within 24 hours regardless of humidity. With an increase in temperature to 33.5°C. the conidia failed to germinate but remained viable for more than 30 hours at a low moisture stress. Under this condition (33.5°C.) the conidia were killed within 30 hours in a high moisture stress.

EFFECT OF LEAF AND FRUIT MATURITY ON GRAPE POWDERY MILDEW.—Viala (31) in 1893 reported that powdery mildew developed very little as the vines matured. He considered this a consequence of lignification and disappearance of chlorophyll from small branches, since other conditions were favorable. Bioletti

TABLE 2.—*Development of grape powdery mildew on single intact leaves subjected to different temperatures and moisture stresses in the controlled atmosphere wind tunnel*

Temperature °C.		RH	VPD	Exposure time	Germination	Infection	
Leaf	Air	%	mm. ± 1	days	%	%	Growth
25.5 ±	27 ± 1	20–25	21	3	70	50	Primary hyphae over 1000μ
27 ±	29 ± 1	15–20	25	5	70	50	Conidial production
29.5 ± 0.5	31.5 ± 0.5	10 ± 1	31	9	13	10	Conidiophore formation
31 ± 0.5	33 ± 0.5	10 ± 1	34	2	40	15	Primary hyphae 250μ
31.5 ± 1	32 ± 1	99 ± 1	1	2	40	15	Primary hyphae 250μ
31.5 ± 0.5	33 ± 0.5	10 ± 1	34	4	20	0	None[a,b]
32 ± 0.5	34 ± 0.5	10 ± 1	36	1	30	0	None[a,c]
32.5 ± 1	33 ± 1	99 ± 1	1	1.25	40	0	None[a]
32.5 ± 0.5	33 ± 0.5	99 ± 1	1	3	15	0	None[a]
33.5 ± 0.5	34 ± 0.5	99 ± 1	1	1.25	0	0	None[d]
33.5 ± 0.5	35 ± 0.5	9 ± 1	38	0.5	0	0	None[c,e]
33.5 ± 0.5	35.5 ± 0.5	10 ± 1	39	2	0	0	None[a,f]

[a] Conidia not viable after this exposure when tested under more favorable temperatures.
[b] Two-day colonies grew about 300μ when exposed to these conditions for 48 hours.
[c] Conidia that were germinated on leaves in the laboratory failed to cause infection under these conditions.
[d] 40 per cent of the conidia were viable after this exposure.
[e] 3 per cent of the conidia were viable after this exposure.
[f] Under these conditions 3-day colonies did not grow during, but remained viable after, 18 hours' exposure. Four-day colonies were killed by a 2.5 days' exposure.

(3) in 1907 reported that after the grapes had lost the green color due to presence of chlorophyll in the skin and had commenced to ripen, they were not attacked by powdery mildew.

Poor development of the fungus on mature leaves (2 months old) was noted under both field and greenhouse conditions. To test this observation, healthy potted plants from the greenhouse and field were brought into the laboratory nursery and inoculated. Celloidin strippings from a series of newly opened to 2-month-old leaves were studied periodically. No significant difference in germination was found on leaves of different ages, but infection, growth, and sporulation were in an inverse proportion to the leaf age. No infection took place on 4 mature leaves more than 2 months old from the field. A representative comparison of development for a period of 2 days after inoculation is as follows: on leaves up to a week old—60 per cent germination, 40 per cent infection, and radial growth to 300 μ occurred; on leaves 2 months old—60 per cent germination, 10 per cent infection, and 150 μ radial growth took place.

Though growth and sporulation are poor on mature leaves in the field, abundant cleistothecial production has been consistently observed on these leaves in California in the fall. This type of mature plant resistance has been attributed, for other powdery mildew fungi (2, 10, 13, 16), to a thickened cuticle.

Light influenced host susceptibility in a few tests. The fungus grew normally on etiolated leaves produced when a plant was enclosed for 3 weeks in a dark chamber. The fungus also grew and sporulated readily on the older leaves of plants previously subjected to darkness for 2 and 3 weeks. Comparable leaves on check plants from the greenhouse resisted growth and sporulation.

New infections on ripe grape fruits have never been observed in the field, but it was not known at what stage of maturity the berries became resistant. Berries of different maturities from the *Vitis vinifera* varieties Muscat of Alexandria, Tokay, Carignane, Thompson Seedless, and Malaga were brought into the laboratory and washed in distilled water. The stem of each excised berry was placed in a vial of water, and the berry was inoculated. As a check, leaves were inoculated at the same time. Four to 6 days after inoculation celloidin strippings and refractive index readings of the expressed juice were made from each berry. The strippings were examined microscopically for the presence or absence of infection. The refractive index, an indication of total soluble solids expressed in approximate percentage of sugar, is a standard field test for maturity of the berries. An Abbe Zeiss refractometer was used for these determinations.

More than 430 individual berries were tested and in all varieties examined, except Malaga where insignificant numbers were tested in the low range, infection and growth decreased to none above a refractive index of 1.3450 or about 8 per cent sugar. Berries were consistently susceptible to infection at a more immature state as indicated by a sugar percentage below 6. Once infection was well established, the fungus continued to sporulate as the berry matured to a sugar content of at least 15 per cent. (This is a refractive index of 1.3600 or above.)

The lack of infection on grapes with a sugar content

above 8 per cent may prove to be a useful tool in the practical control program, for the resistance of berries is correlated with factors developed with maturity. This may be due to the increased sugar, cuticle thickness, or some other factor. Further study is needed to determine the mechanism of resistance of mature grapes to powdery mildew development.

GERMINATION OF POWDERY MILDEW CONIDIA REMAINING IN CHAINS.—In 1923 Yossifovitch (39) made a drawing of a chain of 3 grape powdery mildew conidia in which each of the conidia was germinated. Frequently in this study detached chains have been seen which contained up to 5 conidia in a chain germinating (Fig. 3a). Two tests were made of the germination of conidia in chains of different lengths. Detached leaves were inoculated and floated on tap water in petri dishes. Abundant sporulation occurred over a period of 3 weeks after which time the leaves were inverted over dry slides and tapped lightly to release the chains of conidia. These conidia were incubated 12 hours at 24°C. before germination counts were made.

Observations of the longer chains during the incubation period revealed that germination of the spores proceeded rapidly from one end of the chain in some cases up to about 6 spores, but seldom were continuous chains of germinated spores longer than this. Observations after 24 and 48 hours revealed that little or no additional germination occurred after the 12-hour readings, even in chains of 10 turgid conidia containing only 5 which had germinated. Frequently there were skips where 1 or more non-germinated conidia were found between those that had germinated.

There was a decrease in germination as the length of the chain increased (68 per cent for single conidia to 41 per cent for conidia in chains of 10). No significant difference in germination of terminal conidia over that of internal conidia was noted. This was perhaps due to the fact that germination proceeded from one end of the chain to over 5 conidia within the chain. Never in this study have the attached conidia in chains on the leaf (*in situ*) been observed to be germinated. In 1886 Smith (27) described and illustrated germination *in situ* for the grape powdery mildew.

These observations may be used in support of Brodie's (4,5) explanation of the physiology of spore germination and the lack of conidial germination *in situ*, but they offer an additional question. In this study the skips or non-germinated spore at either end of a series of germinated spores would be difficult to explain by a gas exchange theory. Brodie's ideas are based on the exchange of O_2 and CO_2 through the permeable papilla which is exposed when conidia are detached from the chain *in situ*. Previous experimental evidence and observations (5,6) do not preclude the possibility that a growth inhibitor, or more specifically, germination inhibitor, may be supplied to the conidia in chains through the conidiophore from the parent fungus mycelium.

DISCUSSION AND CONCLUSIONS.—The cardinal temperatures for spore germination, below 6°C., about 25°C. and 34°C., were broader than for infection and development, which began very slowly at 7°C. and did not occur at leaf temperatures above 31.5°C. Rapid spore germination and mycelial growth took place over a range of 21–30°C. Leaf temperatures above 31°C. killed the conidia and mildew colonies. Thus grape powdery mildew is capable of developing at temperatures below those favorable for grape plant growth but is killed at high temperatures which do not apparently harm the host plant. These conclusions are generally in line with those of Yarwood (38) and Yossifovitch (39).

Leaf temperatures were used in this study of the growth and development of powdery mildew because the air temperatures used in field observations can vary from 5–10°C. above or below the temperature of leaves in the vineyard, depending on the intensity of radiation to which they are exposed. Thus the cardinal temperatures reported by the author cannot be accurately compared with those previously reported from field observations. The lethal effect of higher temperatures, worked out by the author, is considerably buffered by the host plant. At higher temperatures the transpiring leaves can reduce the temperature of the air within the foliage, and the inside of a tight cluster of grapes has a time lag in warming up to this temperature. Thus, in short periods where the temperature goes as high as 40°C., the colonies exposed to sunlight and the full effect of the high temperature, would be rapidly killed. The colonies on cluster stems protected by cool berries under the foliage would not be subjected to these temperatures and would survive. The powdery mildew fungus might be completely destroyed at air temperatures over 35°C. lasting for extended periods in the early part of the growing season, when the berries were yet small and foliage was sparse.

The humidity values were usually given in this paper as vapor pressure deficit (VPD) rather than relative humidity (RH). This was done because it is felt by the author that the atmospheric humidity functions chiefly to regulate the evaporation or moisture stress. A measure of this quality of the atmosphere can be determined from RH values only indirectly and when the temperature is known by converting them to VPD. The evaporation power or moisture stress of space is expressed by VPD independently of temperature, but the response of an organism to a given moisture stress may be largely determined by the temperature. Consequently temperature readings should accompany humidity readings, RH or VPD.

Spore germination on dry glass slides at temperatures up to the optimum for germination was not influenced by low humidity (up to 24 mm. VPD). Above 27°C. low humidity caused a slight but significant reduction in germination. Conidial germination did take place at the highest moisture stress possible (38 mm. VPD), which was at the highest temperature at which germination occurs under any moisture condi-

tion (33°C.) and is twice as great as the moisture stress at 0 per cent RH, 21°C. On the host plant the low humidities are buffered by transpiration moisture gradients, and germination was normal under most severe moisture stresses.

The study of infection and development of the fungus on grape leaves under different humidities at different temperatures revealed that humidity has little or no influence. Normal development occurred at both high and low moisture stresses, and never in this study was the lack of infection or growth attributable to low humidity. These studies emphasize the extreme tolerance of some of the powdery mildew fungi to low moisture conditions as pointed out by Yarwood (34).

Free water causes poor and abnormal germination of conidia and may wash the conidia and mycelium from host tissue. Observations of the action of water on conidia indicate that it is taken into turgid conidia, probably due to the high osmotic pressure of the spore contents, until plasmoptysis occurs, i.e., bursting due to excessive turgor pressure (Fig. 3b). Moisture, then, acts in an inhibitory way only when it is in an excess, i.e., free water.

The author suspects that in most reports of increased mildew in areas of high humidity the temperature was more favorable (lower) in the humid locations. This may well have been the case in the experiments of Yossifovitch (39). Of the 2 important environmental factors which influence the development of fungi (temperature and moisture) temperature is by far the more important to the development of grape powdery mildew.

The results of the experiments on the influence of humidity on conidial germination on glass slides (Fig. 4) revealed an unusual phenomenon. At temperatures of 29°C. and above, a moisture stress between 8–12 mm. VPD caused a slight decrease in germination. Increasing the moisture stress did not continue to cause a significant decrease in germination. This drop at about 10 mm. VPD might be tied to the availability of moisture to a small number of conidia which require additional moisture for germination. Conceivably there are viable conidia in a population which lack sufficient moisture for germination under high temperatures. If this is not the case, possibly the temperatures above 28°C. cause an increased permeability of a few physiologically different conidia which allows them to lose water before germination can occur. These conidia would be contrasted to the majority which contain sufficient moisture and are capable of retaining it under severe stresses until germination is accomplished. This latter characteristic, for other powdery mildew conidia, has been discussed by Corner (10), Brodie (4), Clayton (9), and Yarwood (36). In a humid atmosphere (10 mm. VPD or less) the low moisture conidia probably take enough water from the atmosphere to make them capable of germination. Yarwood (35, 36) found that spores of fungi other than powdery mildews took up water so that their composition was about 70 per cent water at germination in water, and that many mildew conidia normally contain from 65–75 per cent water. This is in agreement with one determination for *U. necator* by the author and contributes evidence for the above-mentioned ideas. There is considerable evidence in the literature that spores of some other powdery mildews will germinate at high moistures stresses (5, 34), but that some will not tolerate low humidities (2, 20). This fact is an indication of a possible phyllogenetic trend away from fungi with moisture requirements which allow only an endophytic existence. The powdery mildew group, Erysiphaceae, may have become adapted to an epiphytic type of growth and resistance to desiccation in the absence of free water or a low moisture stress. The substomatal mycelium of the genera Phyllactinia and Leveillula may be remnants of an endophytic existence.

There is some evidence of differences in moisture requirements for infection and development among the Erysiphaceae. Longree (20) obtained poor development of *Sphaerotheca pannosa* on young rose leaves at 21–22 per cent RH at 21°C. (15 mm. VPD). She found very low spore germination on dry slides at humidities below 95 per cent RH at 21° and 25°C. (about 1 mm. VPD). Yarwood (34) found in his work on *Erysiphe polygoni* a slight decrease in mycelial growth on excised leaves over sulfuric acid at 22°C. and no growth at 27°C. as compared with good growth on excised leaves over water.

In this study of grape powdery mildew very little or no effect on infection and development could be found by changing the moisture stress from 1 to 34 mm VPD. This would indicate that *U. necator* represents the extreme in a group of fungi which show a tolerance to low atmospheric humidity.

These studies indicate that temperature is the primary factor which limits the growth of powdery mildew on grapes. The moderate temperatures of the coastal valleys of California favor its development. Periods of high temperature probably arrest the development of mildew during mid-summer of most seasons in the interior valleys. In the Imperial and Coachella Valleys where the summer temperatures are high for long periods, powdery mildew is not considered an annual problem on grapes.

Department of Plant Pathology
University of California
Davis, California

LITERATURE CITED

1. Anderson, D. B. 1936. Relative humidity or vapor pressure deficit. Ecology **17**: 277-282.
2. Berwith, C. E. 1936. Apple powdery mildew. Phytopathology **26**: 1071-1073.
3. Bioletti, F. T. 1907. Oidium or powdery mildew of the vine. Cal. Agr. Exp. Sta. Bul. **186**: 315-350.
4. Brodie, H. J. 1942. Protoplasmic continuity in the powdery mildew Erysiphe graminis D.C. Canad. Jour. Res. Ser. C. **20**: 595-601.
5. Brodie, H. J. 1945. Further observations on the mechanism of germination of the conidia of various

species of powdery mildew at low humidity. Canad. Jour. Res. Ser. C. **23**: 198-211.
6. Brodie, H. J., and C. C. Neufeld. 1942. The development and structure of the conidia of Erysiphe polygoni D.C. and their germination at low humidity. Canad. Jour. Res. Ser. C. **20**: 41-61.
7. Capetta, G. B. 1951. Ricerche sulle condizioni ecologiche per l'insorgere dell 'epidemia da oidio nella vite dell 'Oltrepo paverse. Ist. Bot. Univ. Pavia, Atti. Ser. 58, **5**: 231-242.
8. Cherewick, W. J. 1944. Studies on the biology of Erysiphe graminis D.C. Canad. Jour. Res. Ser. C. **22**: 52-86.
9. Clayton, C. N. 1942. The germination of fungus spores in relation to controlled humidity. Phytopathology **32**: 921-943.
10. Corner, E. H. J. 1935. Observations on resistance to powdery mildews. New Phytol. **34**: 180-200.
11. Gastella, F. de, and C. C. Brittlebank. 1923. Oidium of the vine. Uncinula spiralis (Berkeley and Cook). Jour. Dept. Agr. Victoria **21**: 673-675, 738-745.
12. Gladwin, F. E. 1928. Downy and powdery mildews of the grape and their control. N. Y. Agr. Exp. Sta. Bul. 560.
13. Graf-Marin, A. 1934. Studies on powdery mildew of cereals. Cornell Agr. Exp. Sta. Memoir **157**: 1-48.
14. Hartmann, H. T., and L. R. McKinnon. 1943. Environment control cabinets for studying the inter-relation of temperature and photoperiod on the growth and development of plants. Amer. Soc. Hort. Sci. Proc. **42**: 475-480.
15. Hashioka, Y. 1937. Relation of temperature and humidity to Sphaerotheca fuliginea (Schlect.) Poll. with special reference to germination, viability and infection. Nat. Hist. Soc. Formosa, Trans. **27**: 129-145.
16. Hashioka, Y. 1938. The mode of infection by Sphaerotheca fuliginea (Schlect.) Poll, in susceptible, resistant and immune plants. Nat. Hist. Soc. Formosa, Trans. **28**: 47-60.
17. Hepburn, J. R. I. 1927. The vapor pressure of water over sulfuric acid-water mixtures at 25°C. and its measurement by an improved dewpoint apparatus. Phys. Soc. London Proc. **40**: 249-260.
18. Jacob, H. E. 1929. Powdery mildew of the grape and its control in California. Cal. Agr. Ext. Ser. Circ. 31.
19. Lindley, J. 1852. Vine mildew. Gardener's Chron. **12**: 389.
20. Longree, Karla. 1939. The effect of temperature and relative humidity on the powdery mildew of roses. Cornell Agr. Exp. Sta. Memoir 223.
21. Lorenzen, C., Jr. 1949. The thermocouple in agricultural research. Agr. Eng. **30**: 275-279.
22. Mares, H. 1856. Mémoire sur la maladie de la vigne. Soc. Agr. Herault, Bul. 203-218 and 304-310.
23. Mohl, H. von 1853. Uber die Traubenkrankheit. Bot. Zeit. **11**: 385-595.
24. Quaintance, A. L., and C. L. Shear. 1907. Insect and fungus enemies of the grape east of the Rocky Mountains. U. S. Dept. Agr. Farmers' Bul. 284.
25. Ramsay, J. A., C. C. Butler, and J. H. Sang. 1938. The humidity gradient at the surface of a transpiring leaf. Jour. Exp. Biol. **15**: 255-265.
26. Shaw, L. 1935. Intercellular humidity in relation to fire-blight susceptibility in apple and pear. Cornell Agr. Exp. Sta. Memoir 181.
27. Smith, W. G. 1886. Vine Mildew, Oidium tuckeri. Gardener's Chron. **25**: 619-622.
28. Stevens, N. E. 1916. A method for studying the humidity relation of fungi in culture. Phytopathology **6**: 428-432.
29. Thut, H. F. 1939. The relative humidity gradient of stomatal transpiration. Amer. Jour. Bot. **26**: 315-319.
30. Uppal, B. N., G. S. Chemma, and M. N. Kamat. 1931. Powdery mildew of the grape and its control in Bombay. Bombay Dept. Agr. Bul. 163.
31. Viala, P. 1893. Les maladies de la vigne. Montpellier and Paris.
32. Wenzil, H. 1939. Die Untersuchung epiphytischer Pilze nach dem Abdruckverfahren (Zelloidinhautchenmethode.) Abl. Bakt. Abt. 2,c 14-17, 327-342. (Abs. in Rev. Appl. Myc. **19**: 33. 1940.)
33. Wilson, R. E. 1921. Humidity control by means of sulfuric acid solutions with critical compilation of vapor pressure data. Jour. Ind. and Eng. Chem. **13**: 326-331.
34. Yarwood, C. E. 1936. The tolerance of Erysiphe polygoni and certain other powdery mildews to low humidity. Phytopathology **26**: 845-849.
35. Yarwood, C. E. 1950. Water content of fungus spores. Amer. Jour. Bot. **37**: 636-639.
36. Yarwood, C. E. 1952. Some water relations of Erysiphe polygoni conidia. Mycologia **44**: 506-522.
37. Yarwood, C. E., and W. E. Hazen. 1944. The relative humidity at leaf surfaces. Amer. Jour. Bot. **31**: 129-135.
38. Yarwood, C. E., S. Sidky, M. Cohen, and V. Santilli. 1954. Temperature relations of powdery mildews. Hilgardia **22**: 603-622.
39. Yossifovitch, M. 1923. Contribution à l'étude de l'oidium de la vigne et de son traitement. Univ. Toulouse. Thése Doct.

BACTERIAL STEM ROT AND LEAF SPOT OF PELARGONIUM[1]

Donald E. Munnecke

SUMMARY

Bacterial stem rot and leaf spot of geranium, *Pelargonium hortorum* Bailey, caused by *Xanthomonas pelargonii* (Brown) Starr & Burkholder, has severely limited the production of cuttings from field-grown geraniums in California for several years. The bacterium may cause leaf spots or angular necrotic sectors on leaves of some varieties as well as a black stem rot. No commercial variety of *P. hortorum* was found to be immune from the stem rot phase, but several varieties were resistant to the leaf spot stage of the disease. Several unknown varieties of ivy geranium (*P. peltatum* Ait.) were susceptible, but the Martha Washington pelargonium (*P. domesticum* Bailey) was resistant. The vascular-inhabiting bacteria are carried over in infested cuttings taken from symptomless infected plants, from obviously diseased plants, or in recently infested soil. The bacteria may carry over for at least 3 months in moist field soil. They are extremely infectious and may be disseminated in the field by contact and splashing water, but primarily by contaminated cutting knives. Infected cuttings serve as inoculum for adjacent healthy cuttings in the rooting bed. Field control measures must be based upon establishing disease-free planting stock, using 1-year rotated fields, and following strict sanitary measures. Such a program is presently underway in several commercial establishments in California.

Bacterial stem rot of geranium, *Pelargonium hortorum* Bailey, caused by *Xanthomonas pelargonii* (Brown) Starr & Burkholder has for several years limited production of cuttings from field-grown geraniums in California. The disease reached severe epidemic proportions in the spring and summer of 1952 following an unusually wet season. Since 1952 it has caused field losses of 10–100 per cent (Fig. 1,A), depending upon the variety infected. It is not restricted to California fields; many growers of potted geraniums in the United States have reported severe losses and it has recently been reported severe in Denmark. In 1949, of the more than 25 million unfinished geranium plants and cuttings grown in the United States, over 2 million were grown in California (2). Since geraniums are the leading potted-plant crop in the United States, the disease has become of considerable economic importance.

Various bacterial pathogens have been reported on geranium (*P. hortorum*) causing either a leaf spot or a stem rot. The stem rot stage was reported in France (11) and in the United States in 1890 (5) and 1912 (6), but no investigations or inoculations were made. Lewis (9) described a bacterial leaf spot of *Erodium* and *Pelargonium* which he named *Pseudomonas erodii*. The first report of geranium leaf spot based upon a study of the causal agent and inoculation tests was made by Brown (1) in 1923. Her isolate was different from Lewis'; the incubation period was shorter, the spots were smaller and more localized, and the cultures did not produce a green fluorescent pigment. She named the causal agent *Bacterium pelargoni*. No mention of the stem rot phase was made. In 1932, Dodge and Swift (4) produced the stem rot stage on 4 of 7 geranium plants inoculated with bacteria isolated from leaf spots, but they did not conclude that the 2 symptoms were caused by the same organism. This was still undecided in a later publication (3). Hildebrand and Yezak (8) recently have concluded that *Pseudomonas erodii* was distinct from *Xanthomonas pelargonii*. The first thorough integrated study of the leaf and stem rot of Pelargonium was made in 1952 by Hellmers (7). He definitely showed that the leaf spot and stem rot stages were due to the same organism. The work reported in this paper has been briefly reported (10), was done concurrently with Hellmer's, and substantiates his results. He was concerned with Danish varieties in the greenhouse, whereas this report covers the field aspects of the disease in the United States.

SYMPTOMS.—*X. pelargonii* attacks leaves, stems, and cuttings of *P. hortorum*. All the common commercial varieties are susceptible to the stem rot stage, whereas some are resistant to the leaf spot stage. This may be the reason for the long delayed correlation of the 2 symptoms. Symptoms on leaves, stems, and cuttings are as follows:

Leaf symptoms.—Infected leaves either produce round leaf spots (Fig. 1,C; 2,A) or large, angular dead areas (Fig. 1,D), depending upon the variety. The leaf spot has been most commonly reported, but it is not the most common symptoms in California. First leaf spot symptoms appear as small water-soaked spots on the underside of the leaf. In 2–3 days the spots become well defined, slightly sunken, and about 2–3 mm. in diameter. They rarely coalesce. This stage is rapidly followed by necrosis and wilting of the leaf, which abscises or droops and hangs on the plant for a week or 2. The original spots turn dark brown to black and become hard and dry. If there is no secondary spread of the bacteria, the other leaves on the plant do not bcome spotted. Rather, the bacteria spread through the vascular system of the petiole downward to the stem and then back up to the top of the plant. The plant eventually succumbs to the stem rot (Fig. 1,B).

Plants exhibiting the second leaf symptom wilt at the margins of the leaf blade, and the petiole initially remains turgid (Fig. 1,D; 2,C). Infected areas of

[1] Accepted for publication June 12, 1954.

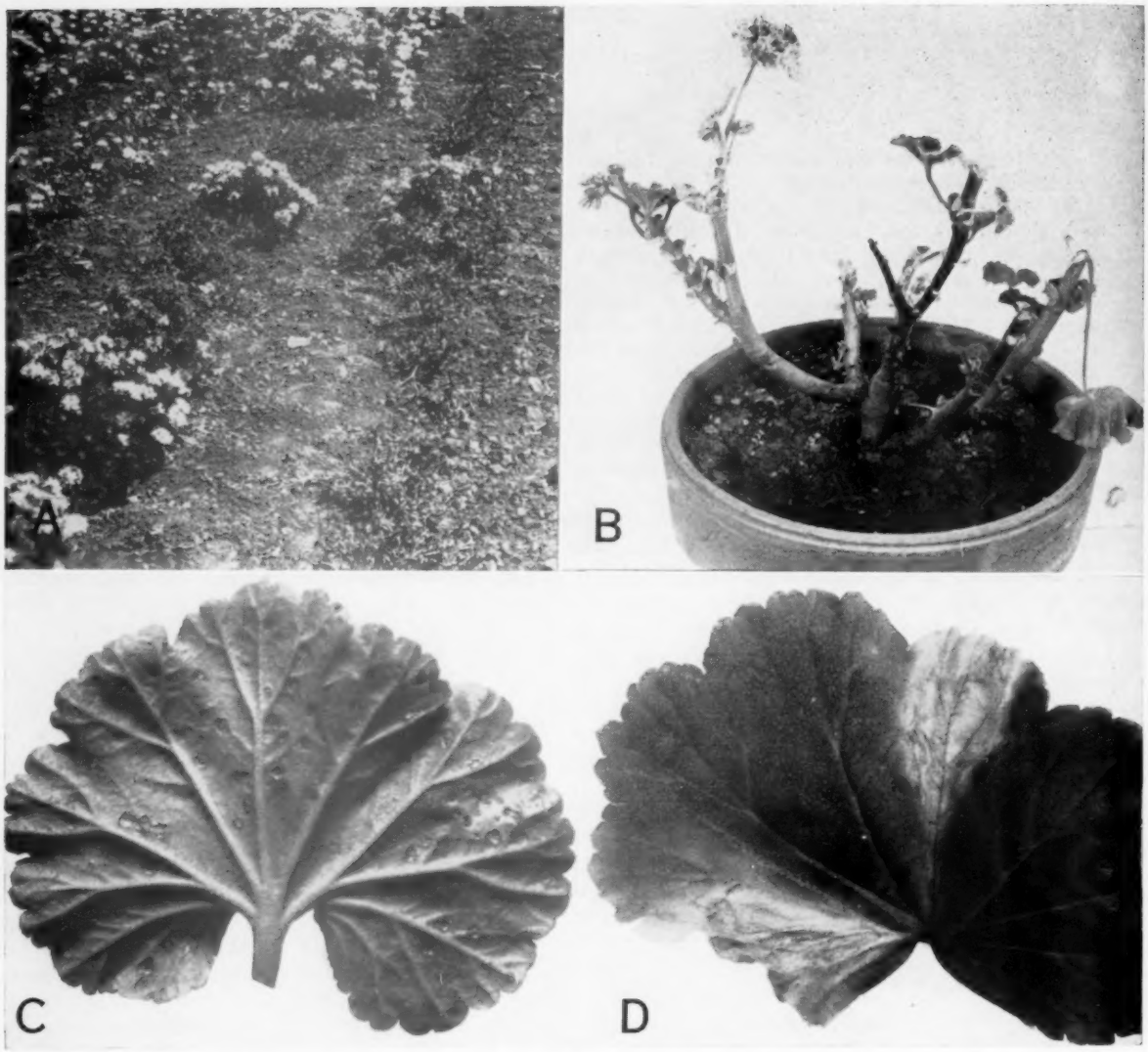


FIG. 1. A) Geranium field with 50–100 per cent loss due to bacterial stem rot. B) Terminal stage of bacterial stem rot. C) Leaf spot symptom. D) Angular necrosis and wilt symptom. Photos by B. B. Markley.

the leaf rapidly become necrotic in angular areas bounded by the veins. Angular necrosis of this type on geraniums is common and may be caused by several other factors, but the flaccidity of the leaf is always associated with bacterial infections. Leaves rapidly abscise, or hang on the plant, as in the case with leaf spot symptoms (Fig. 2,B). After the leaves abscise, the vascular cylinders of the stems may be discolored, although often indistinctly. Angular dead areas and wilting have been produced experimentally on such varieties as Improved Ricard, Red Fiat, Salmon Supreme, and Madam Buchner by spraying bacterial suspension over the leaves.

Stem symptoms.—The stem rot symptom, the one most noticed in California fields (Figs. 1,A; 1,B), has given rise to the term "black rot", commonly used by growers. Vascular bundles in stems and branches of plants infected with the bacteria become brown to black 2–4 weeks after inoculation. At this stage 1 or more leaves on a branch will usually wilt and exhibit the angular necrotic symptom. In a week or 2 the bacteria infect the pith and cortex of the stem, causing a black, sharply limited discoloration. At this stage the exterior portions of the stem are gray and dull in appearance and only the terminals of the branches are left with leaves. The stem rapidly blackens and shrivels into a dry rot, leaving the fibers and epidermis intact and the rest as a disorganized non-viscous mass. At this stage, which is from 4–6 weeks after inoculation, the plant consists of almost completely defoliated, blackened branches with only a few tufts of leaves on the tips. The roots are blackened but not decayed, and the plants are often difficult to uproot. Occasionally a plant will partially recover and produce new, healthy-

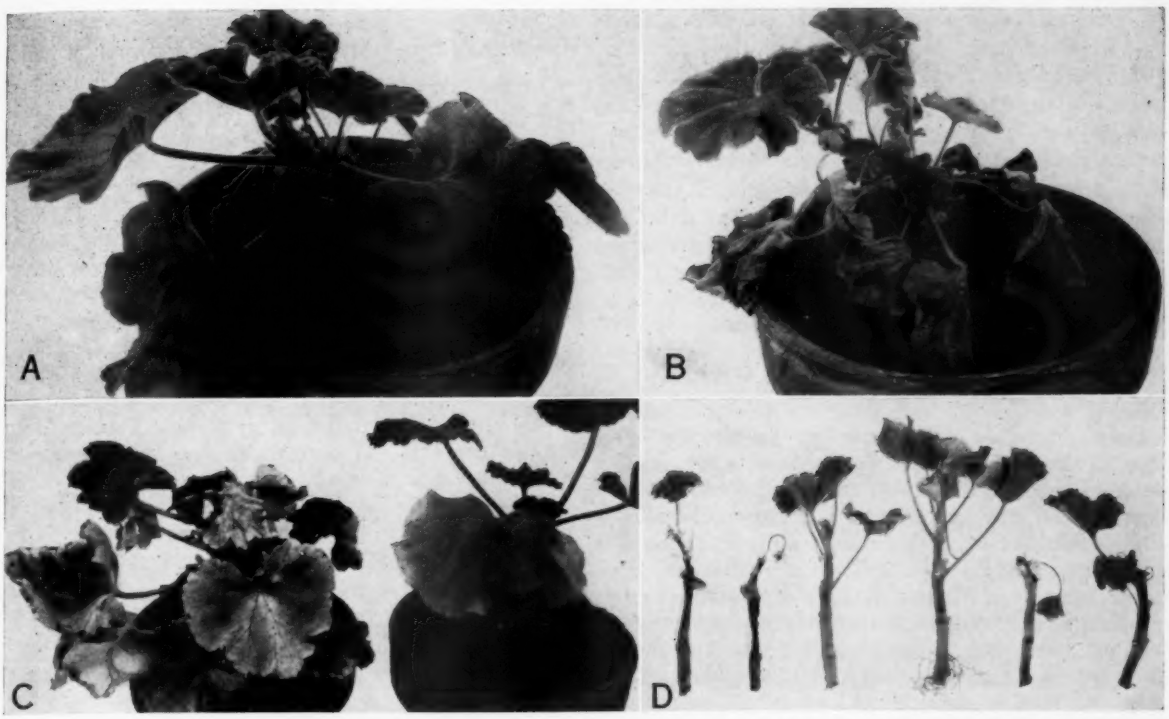


FIG. 2. A, B). Course of development from leaf spot to early stem rot stage. A) Radio Red 14 days after bacteria were rubbed on leaves. First symptoms were noted 8 days after inoculation. B) Same plant 22 days after inoculation. Most of the leaves were killed and the stem was infected. Plant died 3 weeks later. C) Varietal reactions to leaf infection. Plants inoculated by spraying bacteria on the leaves. Radio Red (left), leaf spot; Red Fiat (right), angular necrosis and wilt, but no spots. D) Cutting rot. Disease spread from infected cuttings to adjacent non-infected cuttings through the rooting medium. Photos by B. B. Markley.

appearing branches. However, in 3–6 months these branches usually will die.

Cutting symptoms.—Cuttings infected with the bacteria fail to root, and slowly rot from the base upward (Fig. 2, D). The leaves wilt, and often show the typical angular necrosis and wilt symptoms already described. In 2–4 weeks after the cuttings are placed in sand, the stems become dull black-brown, as with the typical stem rot. We have confirmed Hellmers' (7) symptom differentiation between *Pythium* and bacterial cutting rot. Pythium rot progresses rapidly, often killing within a week, and the cuttings have a shiny coal-black, wet appearance, whereas bacterial rot often takes 3–4 weeks and the cuttings are dull black and have a drier appearance.

THE PATHOGEN.—The causal agent, *X. pelargonii*, may be isolated from diseased tissues, and grows on potato dextrose agar as a creamy yellow colony. No culture studies were made with the bacterium, but the identity of the organism was kindly verified by Dr. M. P. Starr. Since the bacteria rapidly become avirulent in culture, they were kept in living plants and reisolated as the need arose. Free-hand sections of diseased stems showed the bacteria to be intercellular in large numbers in the pith and cortex and intracellular in the vessels. As the rot progresses, the bacteria break through the cell walls and large numbers may be found within the disorganized cells. They apparently are unable to digest the fiber cells or the epidermis, since these tissues are usually the only portions of the plant remaining in diseased stems.

The bacteria are extremely infectious, and plants may be inoculated readily by stem puncture or by spraying bacterial suspensions over the leaves. Plants were incubated in a moist chamber for 24 hours after inoculation by spraying, but not if inoculated by stem puncture.

HOST RANGE AND VARIETAL SUSCEPTIBILITY.—Most of this study was confined to commercial varieties of *P. hortorum*, but limited tests were made with *P. peltatum* and *P. domesticum*. A detailed cross-inoculation study will be reported elsewhere of the relationship of *X. pelargonii* and *X. geranii* on *P. hortorum* and 5 *Geranium* spp. None of the Danish varieties used by Hellmers (7) was used. However, our results on species susceptibility agree closely. None of the commercial *P. hortorum* varieties used was immune from stem rot, either in field or in greenhouse tests. In greenhouse inoculations, Radio Red was the most susceptible variety; Pink Fiat and Olympic Red were almost as susceptible; Improved Ricard, Madam Landry, Madam Buchner, and Red Fiat were moderately susceptible; and Salmon Supreme and Better Times were slightly susceptible. Several growers estimated that their most susceptible varieties were Radio Red, Olympic Red, Pink Fiat, Apple Blossom, Irvington Beauty,

Enchantress Fiat, Ruby, and Montmort, whereas the most resistant were Improved Ricard, Red Landry, Red Fiat, Salmon Ideal, Salmon Supreme, and Better Times. In no case has the author seen or heard of an immune variety. Several unknown varieties of ivy geranium (*P. peltatum*) were inoculated with cultures of *X. pelargonii*. The leaves became spotted and abscised, and eventually the stems developed a brown rot. A few unknown varieties of Martha Washington geranium (*P. domesticum*) were inoculated by spray and stem puncture, but none became infected. Although inoculation tests were very limited, it is presumed that the species is immune. Also, healthy rows of *P. domesticum* have been observed in fields adjacent to severely diseased *P. hortorum* plants.

CORRELATION OF THE LEAF SPOT AND STEM ROT STAGES.—Unpublished inoculation studies by L. H. Davis of this department in 1950 indicated that the stem rot and leaf spot symptoms were manifestations of the same disease. This aspect was investigated further. In each of 2 trials, 2 plants of Radio Red, Salmon Supreme, and Madam Buchner were inoculated by spraying a suspension of the bacteria on the leaves, by stem puncture, or by a combination of the 2 methods. The plants were incubated in a moist chamber for 24 hours, allowed to dry off, and placed in the greenhouse. Within 8 days there were pronounced leaf spots on the sprayed Radio Red plants, a few spots on Madam Buchner, and none on Salmon Supreme. No leaf spots were formed on the plants inoculated by stem puncture only, although they all died of stem rot within 3 months. Thus, the presence or absence of the leaf spot is dependent upon the variety attacked (Fig. 2, C). Possibly, strains of the organism may cause this difference, but no differences have been noted among any of the isolates used in this study.

CARRY-OVER OF THE BACTERIA IN SOIL.—Brown (1) wrote that "from all the evidence gathered the organism seems to be one harbored in the soil," although she did not cite the evidence nor state the period she believed the bacteria to be capable of carrying over. Dodge and Swift (4) also mentioned that it was carried over in soil, and Hellmers (7) believed the soil carry-over to be important in Denmark.

Four greenhouse tests were made using Olympic Red, Red Fiat, Madam Buchner, and Better Times. Soil was infested by incorporating approximately equal volumes of chopped, infected geranium stems in steamed nursery soil or by pouring 24-hour broth cultures of the bacteria over the soil surface. Rooted cuttings were planted in soil immediately after it was infested, or the pots were placed in isolation in the greenhouse, watered regularly, and planted 17–22 days later. Usually, the chopped stems were well decomposed by the time of the delayed planting. Sixty-seven cuttings were planted and in no case was there a carry-over if the soil was allowed to stand as little as 17 days. In fact, only 14 rooted cuttings were infected of 65 that were planted in fresh infested soil. These experiments indicated that the soil carry-over was slight, providing that the diseased plant parts were decomposed before planting.

Field experiments are now in progress on the effect of fallowing on bacterial carry-over. In 1 field of heavy loam soil (Yolo series) infested by plowing under infected geranium plants, lots of 72 Radio Red rooted cuttings were planted immediately after plowing and at intervals of 1, 3, 6, and 9 months. The experiments are to continue for 2 years. All the plants were killed by the disease when planted immediately after plowing. Approximately 25 per cent of the plants were infected in the 1-month fallow plots, and less than 5 per cent were infected in the 3-month fallow plot. Unfortunately, a series planted 6 months after plowing was contaminated during rooting, and the results of the other plots are not yet available. Present indications are that the bacteria cannot carry over for much more than 3 months in soil, providing it is kept moist so that the stems and plant debris are allowed to decompose. If cuttings are planted immediately in infested field soil, 100 per cent loss may result.

CARRY-OVER OF THE BACTERIA IN CUTTINGS.—Since the bacteria occur in vascular elements, the disease is most commonly carried over in infected cuttings. Cuttings from obviously infected stems invariably harbor the bacteria and these cuttings often succumb to the cutting rot stage of the disease. Cuttings taken from plants without external symptoms are a more insidious source of the disease. One hundred twenty-two vigorous plants in 6-in. pots were used as stock plants. These were inoculated by carefully puncturing the stem so that the bacteria did not touch any other portions of the plants, or the soil. Plants were spaced on the greenhouse bench and watered carefully to prevent contamination by contact or splashing water. Approximately 10 cuttings were taken at random from each variety immediately before inoculation and at intervals of 3, 9, 23, and 35 days after inoculation. Thus, cuttings were taken from uninoculated plants, from symptomless inoculated plants (4 and 7 days series), and from plants with stem rot symptoms. The cuttings were rooted individually in 3-in. pots and protected from contamination by spacing and careful watering. The results are in Table 1.

Only those cuttings which showed either vascular discoloration or obvious black rot of the stems were considered diseased. These data prove that the disease may be transmitted through the cuttings from mother plants before there are any symptoms, since there were no symptoms on the mother plants when the 3 and 9 day cuttings were taken. Approximately 20 per cent of all the cuttings were infected, there being no difference between those taken at different intervals. Radio Red was the most affected variety and Salmon Supreme and Madam Landry were the least. All but 1 of the mother plants were successfully inoculated by the stem puncture method.

DISSEMINATION OF THE BACTERIA IN CUTTING BEDS.—The rooting bed is an ideal medium for the spread of the pathogen since cuttings are placed within 1-in. of

TABLE 1.—*Transmission of X. pelargonii from inoculated plants to the cuttings taken at various times after inoculation*

Variety	Symptoms on mother plants after: 23 days	54 days	Number of infected cuttings taken: 1 day before inoc.	3 days after inoc.	9 days after inoc.	23 days after inoc.	35 days after inoc.
Red Fiat	13/20	20/20	0/5	2/8	1/8	3/8	1/8
Madame Landry	———	30/30	0/11	1/12	2/12	2/12	0/12
Improved Ricard	13/26	25/26	0/9	2/10	3/10	1/10	2/10
Radio Red	9/15	15/15	0/4	4/8	2/8	2/8	5/8
Salmon Supreme	26/30	30/30	0/11	2/11	2/12	3/12	1/12
Totals	61/91	120/121	0/40	11/49	10/50	11/50	9/50

each other and left from 3 to 6 weeks before being transplanted into the field. The possibility that infected cuttings could contaminate adjacent healthy cuttings was investigated by placing infected cuttings in rows alternately with healthy cuttings and allowing them to root in the usual manner. The results from 1 such trial are in Fig. 2, D. These experiments showed that the bacteria can readily spread from a diseased cutting into the rooting medium and infect adjacent healthy cuttings.

DISSEMINATION OF THE BACTERIA BY INFESTED CUTTING KNIVES.—The most efficient means of transmission of the disease from plant to plant is by the cutting knife. Cuttings are collected from a sequence of plants in the field, and they are usually trimmed before being shipped or used for rooting. After it was experimentally determined that the disease was actually transmissible via the cutting knife, the following disinfectants were used to test the effect on the transmission of the bacteria: mercuric chloride (1:1000), methyl alcohol (70 per cent), trisodium phosphate (10 and 15 per cent), formaldehyde (1 and 5 per cent), copper sulfate (1:1000), lysol (1:200), calcium hypochlorite (1 per cent), agrimycin (100, 200, 400, 800 p.p.m.), and streptomycin sulfate (100, 200, 400, 800 p.p.m.). A scalpel was infested with the bacteria by cutting through a diseased stem, immersed in the disinfectant for 30–60 seconds, the excess liquid shaken off and used in taking a cutting in the usual manner. The process was repeated for each cutting and each cutting was labeled and rooted separately. Thus, the efficacy of the disinfectant was measured on the mother plant as well as on the cutting. The best results have been obtained with mercuric chloride and methyl alcohol. At present, mercuric chloride (1:1000) dip of 60 seconds is recommended for disinfecting the cutting knife. In practice, one knife is left in the disinfectant while another is being used to take cuttings from a plant, and then the knives are exchanged for use on the next plant.

DISSEMINATION OF THE BACTERIA BY RAIN AND PLANT CONTACT.—Field observations indicate rapid spread by rain or overhead watering. The disease may also be spread by plant contact and by farm equipment. It is frequently most severe in the winter months during the rainy season, but this may be due more to the fact that the first crop of cuttings is taken about this time than to a favorable environmental condition.

CONTROL.—The only practical field control of this disease is through the establishment and maintenance of disease-free propagative stock planted on rotated land and grown under strict sanitary procedures. Spraying is useless in the field since the bacteria are within the vessels of the plants and cuttings are continually being taken, thus providing ideal entrance as well as means of dissemination for the bacteria. However, Hellmers (7) reported partial control in the greenhouse by protective sprays of 1 per cent Mercusan or 1 per cent Bordeaux mixture. He emphasized the need for clean stock plants and sterilized soil in conjunction with the spray program. The use of sprays may be feasible in greenhouse operation and should be tried.

Disease-free cuttings were supplied to several growers in the State who planted them in methyl bromide- or chloropicrin-treated ground beds separated from existing fields. Disease-free cuttings from these foundation stocks will be planted in soil which has been out of geranium production for at least 1 year. The cutting knives will be disinfected by immersion in mercuric chloride (1:1000). All cultural operations are being followed to reduce the spread of the bacteria, i.e., plants are watered by furrow irrigation, diseased plants are immediately removed from the field, and cuttings are rooted in freshly sterilized sand. These control measures may serve to eliminate the disease in many California fields.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
LOS ANGELES 24, CALIFORNIA

LITERATURE CITED

1. BROWN, N. A. 1923. Bacterial leafspot of geranium in the eastern United States. Jour. Agr. Res. [U. S.] **23**: 361-372.
2. BUREAU OF THE CENSUS. 1952. Special reports, horticultural specialties. U. S. Census of Agr. 1950, **5** (1): 69.
3. DODGE, B. O., AND H. W. RICKETT. 1948. Diseases and pests of ornamental plants. Ed. 2, Ronald Press Co., N. Y. p. 463-465.
4. DODGE, B. O., AND M. E. SWIFT. 1932. Black stem-rots and leaf-spot of pelargonium. Jour. N. Y. Bot. Gard. **33**: 97-103.
5. GALLOWAY, B. T. 1890. Diseases of geraniums. Jour. Mycol. **6**: 114-115.
6. HEALD, F. D., AND F. A. WOLF. 1912. A plant disease

survey in the vicinity of San Antonio, Texas. U. S. Dept. Agr. Bur. Pl. Ind. Bul. **226**: 86.

7. HELLMERS, E. 1952. Bacterial leaf spot of pelargonium (Xanthomonas pelargonii (Brown) Starr and Burkholder) in Denmark. Trans. Dan. Acad. Tech. Sci. No. 4: 1-40.
8. HILDEBRAND, E. M., AND A. C. YEZAK. 1949. Bacterial leaf spot of pelargonium in Texas. U. S. Dept. Agr. Pl. Dis. Reptr. **33**: 293-294.
9. LEWIS, I. M. 1914. A bacterial disease of erodium and pelargonium. Phytopathology **4**: 221-232.
10. MUNNECKE, D. E. 1953. Bacterial stem rot of Pelargonium hortorum. (Abs.) Phytopathology **43**: 588-589.
11. PRILLIEUX, E., AND G. DELACROIX. 1890. La gangrène de la tige de la Pomme de terre, Maladie bacillaire. Compt. Red. Acad. Sci. **111**: 208-210.

SWEDES NATURALLY INFECTED WITH TWO VIRUSES[1]

G. H. Berkeley and J. H. Tremaine

SUMMARY

Investigations on mosaic diseases of swede in Quebec and Nova Scotia showed that strains of turnip virus 1 and cucumber mosaic virus were involved with the Quebec disease and that strains of turnip virus 1 and cauliflower virus 1 were involved with the disease in Nova Scotia.

Symptoms on a range of 21 hosts, inactivation by heat, and aging at room temperature were used for purposes of identification.

The host range and thermal inactivation points of the 2 turnip virus 1 strains were practically identical but inactivation by aging required 30–36 hours for the Quebec strain and 50–54 hours for the Nova Scotia strain.

Only 10 hosts were susceptible to the cauliflower virus 1 strain, namely swede, rape, cabbage, stock, wallflower, chinese cabbage, chard, radish, Brussels sprouts, and cauliflower. Inactivation of this virus by heat occurred at 78°C. and by aging beyond 6 days.

The strain of cucumber mosaic virus isolated from turnip infected not only some 14 hosts commonly associated with this virus, but also several crucifers including turnip, rape, dames violet, sugar beet, chard, stock, wallflower, and radish. Of these susceptible hosts, dames violet and radish were symptomless carriers whereas turnip sometimes produced extremely mild symptoms or acted as carriers.

Amoeboid inclusion bodies were present in epidermal peelings of *N. glutinosa* or turnip infected with either of the 2 strains of turnip virus 1 isolated from turnip. Moreover, similar inclusion bodies were also present in radish or turnip leaves inoculated with the strain of cauliflower virus 1 isolated from the Nova Scotia disease. No inclusion bodies were found in either *N. glutinosa* or tobacco inoculated with the Quebec strain of cucumber mosaic virus.

Many virus diseases have been reported on cruciferous plants, i.e., turnip, swede, cabbage, radish, broccoli, dames violet, stock, cauliflower, chinese cabbage, horse radish, etc. Single viruses have been reported as the causal agents of most of these diseases. An exception was recorded by Walker, LeBeau, and Pound (5) when they showed that cabbage mosaic in the middle west was caused by a combination of 2 viruses, the cabbage A strain of turnip virus 1 and the cabbage B strain of cauliflower virus 1.

In the fall of 1952 samples of mosaic diseases on Laurentian and Ditmar swedes, *Brassica napobrassica*, were received from the Provinces of Quebec and Nova Scotia, respectively. Since the original transfers from these materials produced symptoms on certain hosts at variance with those encountered with swede mosaic in Ontario (1) further investigations seemed warranted.

This paper reports experiments which showed that 2 distinct viruses were involved in both the Quebec and Nova Scotia diseases.

ISOLATIONS.—*Nova Scotia material.*—On 3 occasions, transfers were made from the original material to *N. glutinosa*, tobacco, swede, and radish. In each test only local lesions developed on tobacco whereas local lesions and systemic infection occurred on *N. glutinosa*, swede, and radish.

The symptoms on these hosts were similar to those associated with turnip virus 1 except for the systemic symptoms on radish. The positive reaction of radish suggested the presence of a second virus in the source material since radish has been reported (1, 2) as insusceptible to strains of turnip virus 1.

That 2 viruses were present in the original material was demonstrated by repeated transfers from 1) single lesions on tobacco and 2) systemically infected leaves of radish. The virus obtained from local lesions on tobacco produced only local lesions on tobacco, and local lesions and systemic infection on *N. glutinosa* and swede, but did not infect radish. This virus is referred to as the Nova Scotia strain of turnip virus 1.

[1] Accepted for publication June 23, 1954.
Contribution No. 1379 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

The second virus obtained via radish did not infect either tobacco or *N. glutinosa* but did infect radish, swede, and cauliflower. The inability of this virus to infect *N. glutinosa* and tobacco and its ability to infect radish, cauliflower, and certain other crucifers indicated that this virus belonged to the cauliflower virus 1 group. This virus is referred to as the Nova Scotia strain of cauliflower virus 1.

Quebec material.—Transfers were also made on 3 occasions from the original Quebec material. In each test, both local lesions and systemic mosaic symptoms developed on tobacco, *N. glutinosa,* and swede. Radish was symptomless. The systemic symptoms on tobacco and *N. glutinosa* were suggestive of cucumber mosaic, thus indicating that 2 viruses were also present in the original Quebec material.

By repeated transfers from single lesions on tobacco and from systemically infected tobacco leaves, 2 viruses were isolated. The one that produced symptoms on tobacco, *N. glutinosa,* and swede similar to those associated with turnip virus 1 is referred to as the Quebec strain of turnip virus 1. The other virus caused symptoms on tobacco and *N. glutinosa* suggestive of those associated with cucumber mosaic virus, and is referred to as the Quebec strain of cucumber mosaic virus.

COMPARISON OF HOST RANGES AND PHYSICAL PROPERTIES.—The isolated viruses were compared with the Ontario strain of turnip virus 1 on a wider host range. The results are given in Table 1.

Turnip virus 1 strains.—The first 3 columns in Table 1 clearly indicate that the Quebec and Nova Scotia strains of turnip virus 1 have much the same host range as the Ontario strain of this virus. All 3 strains produced symptoms similar in type but varying in degree, on swede, rape, cabbage, stock, wallflower, Chinese cabbage, zinnia, *N. glutinosa,* tobacco, petunia, dames violet, spinach, and globe amaranth. Moreover, sugar beets, chard, and radish were insusceptible to all 3 strains. However, though broccoli, Brussels sprouts, kohlrabi, cauliflower, and kale were susceptible to the Ontario strain, only kale reacted with symptoms to the Nova Scotia and Quebec strains. Broccoli was insusceptible to both the Quebec and Nova Scotia strains whereas kohlrabi, Brussels sprouts, and cauliflower were symptomless carriers of these viruses.

Therefore, the Quebec and Nova Scotia strains appear to be identical but differ from the Ontario strain in so far as host range is concerned.

The thermal inactivation points of these viruses were practically identical, namely 59–61°C. for the Ontario strain and 59–60°C. for the Nova Scotia and Quebec strains of turnip virus 1.

Inactivation by aging at room temperature occurred at 54 hours for the Nova Scotia strain, 36–48 hours for the Ontario strain and 30–36 hours for the Quebec strain. These tests were carried out at the same time under identical environmental conditions.

These results indicate minor differences between the 3 strains. Therefore on basis of host range, aging, and thermal inactivation points the Nova Scotia and Quebec viruses fall naturally into the turnip virus 1 group.

Cauliflower virus 1.—Column 4 of Table 1 shows that the host range of the second virus isolated from the Nova Scotia disease was confined to cruciferous

TABLE 1. *Susceptible and insusceptible hosts of viruses isolated from swedes from Quebec and Nova Scotia*

Hosts	Viruses				
	Ont. T.V.1	Que. T.V.1	N.S. T.V.1	N.S. Caul.V.1	Que. Cuc. Mosaic Virus
Swede	+	+	+	+	+
Rape	+	+	+	+	+
Cabbage	+	+	+	+	−
Stock	+	+	+	+	+
Wallflower	+	+	+	+	+
Ch. cabbage	+	+	+	+	−
Zinnia	+	+	+	−	+
N. glutinosa	+	+	+	−	+
Tobacco	+	+	+	−	+
Petunia	+	+	+	−	+
Dames Violet	+	+	+	−	C
Spinach	+	+	+	−	+
Globe amaranth	+	+	+	−	+
Sugar Beet	−	−	−	−	+
Swiss Chard	−	−	−	−	+
Radish	−	−	−	+	C
Broccoli	+	−	−	−	−
Brussels sprouts	+	C	C	+	−
Kohlrabi	+	C	C	C	−
Cauliflower	+	C	C	+	−
Kale	+	+	+	C	−

+ = definite symptoms
− = no symptoms
C = symptomless carrier

plants. This host range is in close agreement with that reported by Tompkins (4) for cauliflower virus 1, except for kale and kohlrabi. Whereas Tompkins reported symptoms on kale and kohlrabi, no symptoms developed on the same varieties of these plants with the Nova Scotia virus. However, the virus was recovered from these plants, indicating that they were symptomless carriers. This would suggest that the Nova Scotia virus is a slightly different strain.

Further evidence that the Nova Scotia virus belongs to the cauliflower virus 1 group was provided by its inactivation by heat and aging. The thermal inactivation point of the Nova Scotia virus was 77–78°C., which is in close agreement with 75°C. as reported by Tompkins. Moreover its viability after 6 days' storage at room temperature also indicates a close relationship with the cauliflower virus 1 group.

Cucumber mosaic virus.—As already pointed out, this virus produced symptoms on tobacco and *N. glutinosa* similar to those associated with cucumber mosaic virus on these hosts. Accordingly, this virus was tested on the hosts listed in Table 1 and on several additional hosts.

The symptoms on tobacco, *N. glutinosa*, garden beet, spinach, zinnia, cucumber, broad bean, cowpea, pea, snapdragon, pepper, petunia, sugar beet, and globe amaranth were, in general, similar to those associated with cucumber mosaic virus on these hosts. In addition, however, mild mosaic symptoms developed on rape, stock, wallflower, and chard, whereas radish and dames violet were symptomless carriers. Though mild symptoms were generally produced on swedes, at times they did not appear; the virus was readily recovered from such symptomless plants. This virus was inactivated in 10 minute exposures to 68°–69°C. and by aging at room temperature for 120 hours. The symptoms, host range, and physical properties of this virus indicate that it belongs to the cucumber mosaic virus group.

Pound and Walker (5) reported that 2 strains of cucumber mosaic virus isolated from dames violet, an ornamental crucifer, were capable of producing pronounced symptoms on dames violet and certain other crucifers. However, the strain isolated from swedes in Quebec did not produce symptoms on dames violet even when inoculated plants were kept longer than 3 months. Other differences between the dames violet and the Quebec swede strains of cucumber mosaic virus should be noted. The swede strain produced recognizable mottle on rape, wallflower, and stock whereas the dames violet strains did not. Moreover, though no systemic symptoms developed on kale, cauliflower, cabbage, Brussels sprouts, and kohlrabi with the dames violet strains, the viruses were recovered from such plants; that is, they were symptomless carriers. The swede strain neither produced symptoms on these hosts nor was recovered from any of them. The swede strain therefore must be considered to be a third strain of cucumber mosaic virus that is capable of infecting certain crucifers.

Inclusion bodies.—Using the phloxine and trypan blue technique, Berkeley and Weintraub (1) found amoeboid inclusion bodies in subveinal epidermis of turnip and *N. glutinosa* leaves infected with the Ontario strain of turnip virus 1. Similar inclusion bodies were found in subveinal epidermal peelings of turnip, and *N. glutinosa* leaves infected with both the Quebec or Nova Scotia strains of turnip virus 1. Also amoeboid bodies of similar structure and size were observed in subveinal tissue of radish and turnip leaves infected with the Nova Scotia strain of cauliflower virus 1. Amoeboid bodies were not observed in *N. glutinosa* or *N. tabacum* leaves infected with the Quebec strain of cucumber mosaic virus, nor were these bodies observed in subveinal tissue from healthy plants of *N. glutinosa*, tobacco, or turnip of the same source and age.

Discussion.—The experimental results reported in this paper reveal for the first time that swedes may be naturally infected with either of 2 combinations of viruses, namely strains of turnip virus 1 and cauliflower 1 or strains of turnip virus 1 and the cucumber mosaic virus. Previously cabbage had been shown to be naturally infected with strains of turnip virus 1 and cauliflower virus 1 (5).

Laboratory of Plant Pathology,
St. Catharines, Ontario, Canada

LITERATURE CITED

1. Berkeley, G. H., and M. Weintraub. 1952. Turnip mosaic. Phytopathology **42**: 258-260.
2. Chamberlain, E. E. 1939. Turnip mosaic, extended host range and identity. New Zeal. Jour. Sci. & Tech. **21**: 212A-223A.
3. Pound, G. S., and J. C. Walker. 1948. Strains of cucumber mosaic virus pathogenic on crucifers. Jour. Agr. Res. [U.S.] **77**: 1-12.
4. Tompkins, C. M. 1937. A transmissible mosaic disease of cauliflower. Jour. Agr. Res. [U.S.] **55**: 33-46.
5. Walker, J. C., F. L. LeBeau, and G. S. Pound. 1945. Viruses associated with cabbage mosaic. Jour. Agr. Res. [U.S.] **70**: 379-404.

HENDERSONULA TORULOIDEA NATTRASS ON CITRUS IN CALIFORNIA [1]

E. C. Calavan and J. M. Wallace [2]

SUMMARY

Hendersonula toruloidea Nattrass was shown to be a wound-invading pathogen capable of causing dieback and cankers on grapefruit trees in southern California. The toruloid form of this fungus is common on *Citrus* species in the Salton Basin and in a few other locations, and the pycnidial stage has been collected on lemon bark.

Pycnospores from lemon bark and from potato-dextrose-agar culture averaged 4.9μ by 12.4μ. Optimum temperature for radial growth is about 33°C. Good growth occurs at temperatures of 18°–39°C.

H. toruloidea occurs frequently, but not consistently, in association with lesions of the Rio Grande gummosis type on grapefruit in the Salton Basin. Natural as well as artificial infections sometimes cause a form of gummosis having a close external resemblance to Rio Grande gummosis, but the internal symptoms of the 2 diseases are different.

Except under certain adverse environmental conditions, control measures for *H. toruloidea* on *Citrus* species are unnecessary.

The purposes of this investigation were to determine the relationships of *Hendersonula toruloidea* Nattrass to branch dieback and canker, and to a gumming condition resembling Rio Grande gummosis on grapefruit in southern California.

Branch blight and gummosis associated with a toruloid fungus similar to *Torula dimidiata* Penzig were observed by H. S. Fawcett on orange and grapefruit trees in California in 1923 (4, 18). After the freeze of 1931 in northern California, A. J. Olson isolated a toruloid fungus from citrus near Hamilton City. He considered this fungus to be similar to *Hendersonula toruloidea* Nattrass, and demonstrated its ability to produce wood rot and gumming on lemon and orange trees (4, 19). Originally *H. toruloidea* was described in 1933 as a new species on deciduous trees in Egypt by Nattrass (10), who later found the same fungus on lemon near Lapithos, Cyprus (11). Penzig's (15) meager description of *Torula dimidiata* on lemon in Italy indicates that a fungus similar to and possibly identical with the toruloid form of *H. toruloidea* has been present in the Mediterranean region for at least 66 years. A branch-wilt disease of Persian walnuts in California was reported in 1945 by Wilson (17), who described the causal fungus in 1947 as *Exosporina fawcetti* Wilson and indicated its apparent identity with certain fungus isolates from lemon and grapefruit bark (18). In 1948 Calavan and Wallace (2) reported that lesions had been produced on Marsh grapefruit trees by inoculations with an isolate of *E. fawcetti* Wilson from grapefruit. Later studies by Wilson (19) revealed that *E. fawcetti* sometimes forms pycnidia and should be designated as *H. toruloidea* Nattrass.

HOSTS AND DISTRIBUTION OF THE FUNGUS.—The known hosts and the distribution of *Hendersonula toruloidea* are as follows: apple, *Malus* sp., in Egypt and possibly in Cyprus (10); apricot, *Prunus armeniaca* L., in Egypt (10) and (by inoculation) in California (19); European chestnut, *Castanea sativa* Mill., in California (18, 19, 20); *Euonymus* sp. in California (20); fig, *Ficus carica* L., in Cyprus (11) and in California (16, 20); grapefruit, *citrus paradisi* Macfadyen, in California (2, 4, 18, 19); lemon, *Citrus limon* (Linn.) Burmann, in California (4, 18, 19), Cyprus (11), and perhaps Italy (15); lime, *Citrus aurantifolia* (Christm.) Swingle, in Arizona and California; mandarin orange, *Cirtus reticulata* Blanco, in California; peach, *Prunus persica* L., (by inoculation) in California (19); plum, *Prunus* sp., in Egypt (10); *Populus nigra* in Cyprus (11); sweet orange, *Citrus sinensis* (Linn.) Osbeck, trees in California (4, 18, 19) and fruit in Southern Rhodesia (1); California black walnut, *Juglans hindsii* Jepson, in California (18, 19); and Persian walnut, *Juglans regia* L., in California (9, 18, 19). Nattrass also has reported *H. toruloidea* on walnut, *Juglans* sp., from Cyprus (11).

In California, this fungus has been found on citrus trees in the northern part of the state near Hamilton City (18), in the central section near Porterville (4, 18, 19), and in the southern part of the state at Elsinore, Hemet, and Riverside on the west side of the San Jacinto Mountains, and at many locations in the Salton Basin (2) on the east side of the mountains. In Arizona, the fungus occurs on citrus near Phoenix. All these locations have hot summers with daily maximum temperatures ranging from 40° to 52°C. *H. toruloidea* has not yet been found on citrus trees in the relatively cool coastal valleys and plains of southern California.

IMPORTANCE.—*Hendersonula toruloidea* on citrus is important chiefly as a wound-invading pathogen. It occurs frequently on frozen or sunburned branches and trunks, and on branches with untreated pruning wounds or other severe mechanical injuries. Pruning wounds treated with a fungicide and an asphalt pruning compound rarely if ever become infection sites for *H. toruloidea*. On grapefruit trees in the Coachella and Imperial valleys the fungus is occasionally found in severe psorosis lesions and frequently in association with a disease closely resembling Rio

[1] Accepted for publication June 15, 1954.
Paper No. 805, University of California Citrus Experiment Station, Riverside, California.

[2] The authors are indebted to Dr. Donald C. Erwin of this station for collecting specimens and to Messrs. Marvin P. Miller and George L. Winright of the California Agricultural Extension Service for valuable aid in collecting specimens and locating experiments.

Grande gummosis of grapefruit in Texas (6, 7, 8, 13, 14). In the Salton Basin of California, damage by *H. toruloidea* is far more important on grapefruit trees than on other citrus species. In some orchards most trees are affected. Sweet orange appears to be more resistant than grapefruit, lemon, or lime. Occasional infection of sweet orange has occurred in central, northern, and southern California (4, 18).

Hendersonula lesions on the trunks of trees from which the tops have been removed during top-working operations may involve large portions of the trunks and cause irreparable damage. This type of damage has occurred on orange trees[3] in Tulare County and on grapefruit trees in Riverside County.

Five-year-old grapefruit trees in an orchard near Elsinore, Riverside County, were top-worked to another grapefruit variety in May, 1952, by a commercial propagator, by means of bark grafts placed in trunks which had been cut off at a height of about 24 to 36 in. No whitewash or shade was provided for the grafted stumps for approximately 1 week after this operation. During this week the weather was clear and hot and many of the unprotected trunks were sunburned. Some of the tardily erected shades were inadequate (Fig. 1, A). *H. toruloidea*, which was present both in an adjacent walnut orchard and in the grapefruit branches, invaded the sunburned areas, penetrated the wood, and overran some of the scions (Fig. 1, B). Vigorously growing shoots from other scions were killed by the advancing fungus (Fig. 1, C). Some of the wounds made in grafting were invaded directly, in the absence of sunburn, probably by hyphae from *Hendersonula* spores introduced during grafting. By mid-August about 30 per cent of the trees in the orchard were ruined or badly damaged.

THE FUNGUS.—The characteristics of *Hendersonula toruloidea* on some of its natural hosts and in culture have been described by Nattrass (10) and by Wilson (18, 19, 20), who included an isolate from citrus in his study (18).

Although the toruloid form of this fungus is the one commonly found on *Citrus* species, the pycnidial form was collected in October, 1951, just above the soil line on frost-damaged bark of Eureka lemon trunks near Holtville in Imperial County. Pycnospores from this collection were of 3 types: hyaline continuous, hyaline to subhyaline uniseptate, and biseptate with the terminal cells hyaline to subhyaline and the middle cell light to dark brown (10, 19). These spores were 3.5–6.0μ wide (average 4.9μ) and 9.8–14.7μ long (average 12.2μ).

Most cultures on agar media produced only arthrospores. However, 1 single-arthrosporic isolate from a citrus root developed a few pycnidia on a potato-dextrose-agar slant in 20 weeks at room temperature (about 15°–35°C.). Biseptate pycnospores from this culture were 4.2–6.1μ wide (average 5.0μ) and 10.4–15.0μ long (average 12.6μ). Measurements of spores from both sources agree rather closely with those reported by Nattrass (10) and Wilson (19).

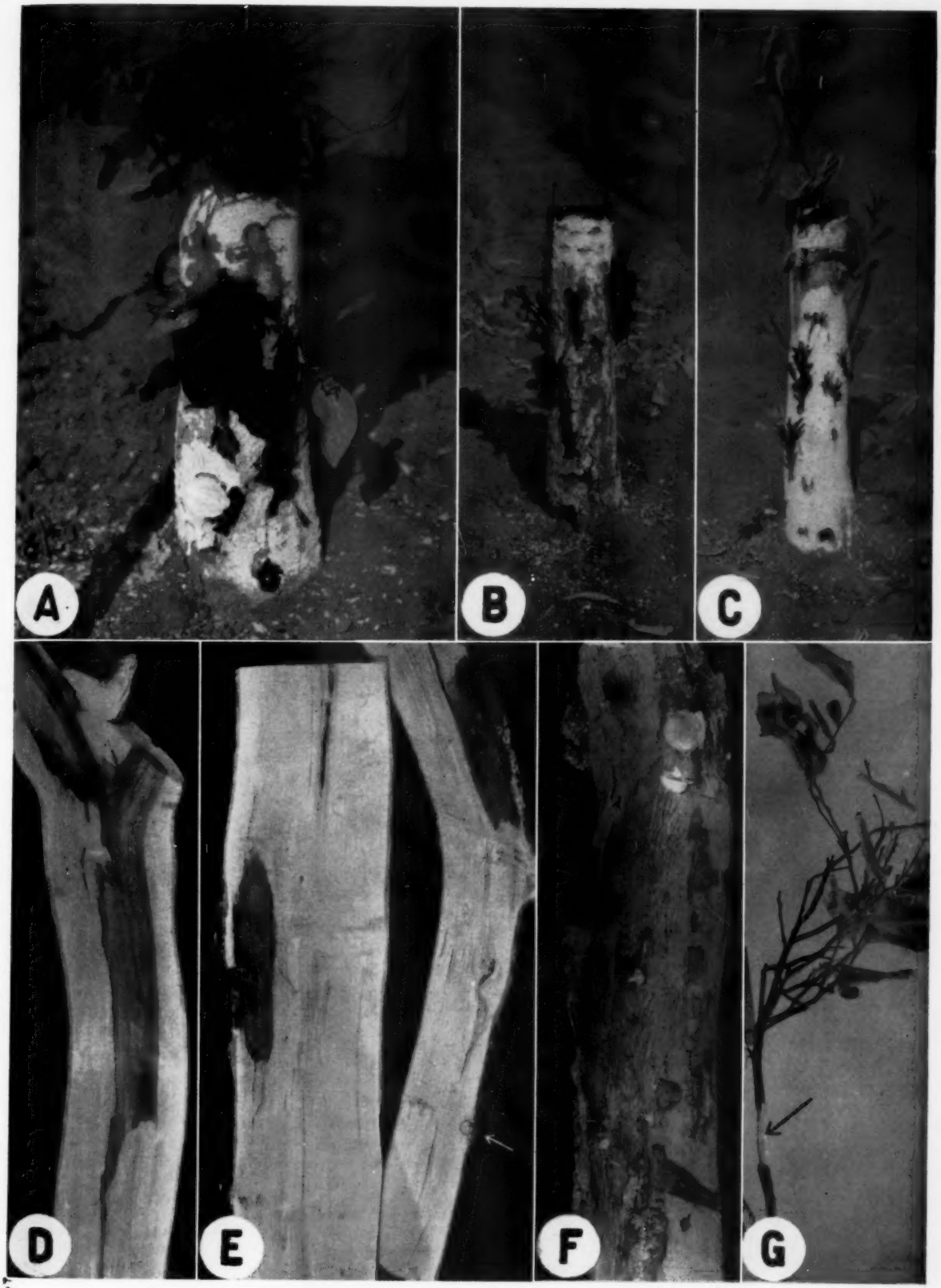
Extent of radial growth at various temperatures of 2 single-arthrosporic cultures on potato-dextrose agar was compared (Fig. 2). Isolate B was obtained from a grapefruit branch and isolate R from an unidentified citrus root about 1 ft. underground. Both specimens were collected near Thermal, California. Six colonies of each isolate, started from water agar discs 5 mm. in diameter and 2 mm. thick, containing germinated arthrospores, were incubated 44 hours at temperatures shown (± 1°C.) on the graph. At 6° and at 12°C. the fungus failed to grow out of the discs during this period. Over the range from 18° to 39°C. the average radial growth of the branch isolate slightly exceeded that of the root isolate. Most rapid growth by both isolates occurred at 33°C., but good growth was obtained at 39°C. At 25°C. radial growth rates of these isolates were slightly greater than that of Wilson's grapefruit isolate but less than that of his grapefruit reisolate (18). Optimum growth occurred at about 33°C., a result agreeing with Wilson's data. Each isolate grew more rapidly at 36°C. than at 30°C., however, and this is a reversal of the trend shown by his isolates.

SYMPTOMS.—Symptoms of *Hendersonula toruloidea* on citrus trees are similar to those described by Wilson (18) on Persian walnut. The most conspicuous features are: 1) formation of a sooty layer of arthrospores between the periderm and the cortex (Fig. 1, A), and 2) deterioration and wilting of branches. Infected citrus branches may wilt during late spring, summer, or early fall, or may gradually defoliate and die. In general, the wilt symptom is much less common in citrus than in walnut. Many infections fail to kill citrus branches. Others progress so slowly that defoliation precedes death. Infection apparently occurs only in injured tissues. Once it has entered the host, the fungus spreads rapidly through both bark and wood. Wood is discolored dark gray to black (Fig. 1, D). As in walnut (18), the older portions of wood near the pith are often killed far beyond the margins

[3] Information from J. C. Johnston, Extension Citrus Specialist, Agricultural Extension Service, University of California, Riverside.

→

FIG. 1. Symptoms of *Hendersonula toruloidea* Nattrass on trunks and branches of grapefruit trees: A) Sooty layer of arthrospores under sloughing periderm of sunburned trunk. B) Infected sunburned trunk with scions killed soon after grafting. C) Infected trunk and wilted shoot of scion overrun by fungus about 3 months after grafting. D) Dark-stained heartwood in a branch with natural infection. E) Dark-stained wood near wound inoculations made 6 months earlier. (Arrow indicates control wound.) F) Gum and bark splits over gum pockets on a branch with natural infection. Photograph by T. A. DeWolfe. G) Wilted twig 6 months after inoculation at arrow. Photograph by L. J. Klotz.


A
B
C
D
E
F
G

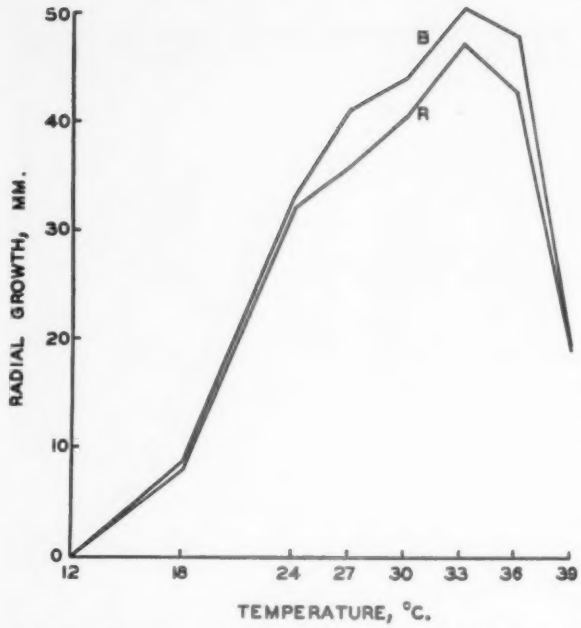


FIG. 2. Extent of radial growth of 2 single-arthrosporic isolates of *H. toruloidea* on potato-dextrose agar in 44 hours. The parent culture of isolate B was obtained from a grapefruit branch; that of isolate R was obtained from a citrus root.

of bark lesions (Fig. 1, D and E). The bark darkens internally in necrotic areas and may become almost black. In many cases a very dark green line appears on the cambial faces of the bark and wood at the lesion margin (4, 18). Usually, the most obvious symptom of *H. toruloidea* on citrus bark is the sloughing of the periderm to expose a layer of powdery, sooty spores (Fig. 1, A) and, sometimes, numerous black stromata. In very small branches, however, and occasionally in large ones, the periderm may remain firmly attached.

Gum exudes from pockets under the bark through openings ranging from minute pores to cracks 1 in. or more in length (Fig. 1, F) near the margins of many lesions (4, 18).

METHODS AND RESULTS OF INOCULATIONS.—Wound inoculations with a pure culture of *Hendersonula toruloidea* were made in 16-year-old Marsh grapefruit trees in 1947 in Imperial County. The first set of 48 inoculations, on April 2 and 3, was made in wounds produced by driving a blunt, heated icepick into the tree at points previously washed with 95 per cent ethanol. Bits of the fungus on potato-dextrose agar were pressed into the wounds. A control wound about 5 in. away from each inoculation received only sterile potato-dextrose agar. Inoculations and check wounds were covered with flamed strips of adhesive tape, the margins of which were sealed to the bark with Duco household cement. Inoculations of this series were evenly divided between an orchard near Calexico and one near Imperial, 12 trees receiving 4 inoculations each. Six of these trees were healthy and vigorous; the other 6 had considerable branch dieback.

Examination of inoculations and check wounds 33 days later revealed gumming around the sites of 44 inoculations. A lesion had developed around each inoculation, including the 4 without external gum. Maximum size of lesions was 4.0 by 2.6 cm. No infection had occurred at check wounds.

At the second examination, 103 days after inoculation, callus tissue was forming around the margins of all lesions except 3. Twenty-two lesions were still gumming. Maximum lesion length was 9 cm. Between July 15, 1947, and January 30, 1948, the average increase in lesion length was about 6 cm. Thirteen months after inoculation, some of the inoculated branches showed more extensive necrosis in the wood than in the bark (Fig. 1, E), which in some instances had completely covered the original wounds. Maximum length of lesions examined was 18 cm.

Twenty-four additional sealed inoculations and 12 noninoculated sealed control wounds were made July 14 and 15, 1947. In general, the July inoculations caused more profuse gum formation than those made in April. Lesions and gum appeared within 6 months at all inoculation sites except one, but none of the control wounds became infected. The fungus killed 11 of 12 inoculated branches less than 1 in. in diameter (Fig. 1, G), but did not kill large branches within 6 months even though necrosis extended downward as much as 28 cm. from the inoculation. Four types of wounds were used in the July inoculations but no important difference in reaction attributable to wound type was observed. In the larger branches the amounts of longitudinal, tangential, or radial lesion growth were approximately in the ratio 4:2:1. In May, 1948 the fungus was recovered easily from discolored wood near the inoculations regardless of lesion size.

In connection with studies of a gumming and wood-staining condition similar to that described as Rio Grande gummosis (12, 13, 14), *H. toruloidea* was placed in 3 types of wounds in Marsh grapefruit trees near Thermal in May, 1952. Bits of agar containing the fungus were inserted in 4 chisel cuts, in 2 holes drilled into the sides of branches or trunks, and in 9 holes drilled lengthwise into the hearts of pruning stubs and their supporting branches. *Diplodia natalensis* Pole-Evans[4] was mixed with *H. toruloidea* in 3 of the inoculations. Gum was present 5 months later near sites of the 3 mixed inoculations and at 8 of the 12 *Hendersonula* inoculations. A small gum pocket formed about 8 cm. from 2 of the *Hendersonula* inoculations. These pockets were not associated with the kind of wood necrosis typical of Rio Grande gummosis as known in Texas, or with the similar gumming disease on grapefruit in the Coachella Valley of California.

The pink or orange-colored borders usually associated with the Rio Grande gummosis type of disease sometimes develop in *H. toruloidea* infections. Orange-

[4] Supplied by T. A. DeWolfe, University of California Citrus Experiment Station, Riverside.

colored lines were found just outside the darkly stained wood around 3 *H. toruloidea* inoculations in Marsh grapefruit in May, 1953, at Riverside when the inoculations were 10 months old.

CONTROL.—*Hendersonula toruloidea* cankers and branch blight generally are not of sufficient importance on citrus in California to justify the expense of preventive spray treatments. For situations and environments where this fungus is likely to cause significant damage, the following control measures are suggested: 1) maintain trees in good vigor; 2) use adequate frost protection; 3) prune out and burn diseased branches (pruning tools should be sterilized after cutting an infected branch, and the cuts should be made several inches below the parts showing stained wood); 4) treat pruning wounds with a fungicidal pruning compound or with a safe carbolineum mixed with or followed by an asphalt pruning compound; 5) whitewash, immediately, all exposed trunks and branches of severely pruned trees.

DISCUSSION.—Comparisons of lesions resulting from *Hendersonula toruloidea* inoculations with lesions typical of Rio Grande gummosis induced by other fungus inoculations and with similar natural infections showed that although certain symptoms of the Rio Grande gummosis type sometimes follow *Hendersonula* inoculations, the typical syndrome (pink or orange lines in the wood, buff-colored foul-smelling wood, gum pockets, and bark blisters) does not develop.

The orange margins occasionally associated with *H. toruloidea* infections are not specific. Similar margins have been seen by the authors in the wood of citrus trees affected by such diseases as dry root rot, psorosis, wood pocket, Rio Grande gummosis (Texas), and in the wood of Marsh grapefruit trees (California) having symptoms resembling Rio Grande gummosis. The darkly stained wood of *Hendersonula* lesions differs greatly in appearance from the buff-colored necrotic wood of Rio Grande gummosis, but does somewhat resemble the darkly stained wood present in the oldest necrotic areas of some Rio Grande gummosis lesions.

Godfrey (5, 6) and Childs (3) found an actinomycete associated with gummosis lesions of grapefruit. In California, actinomycetes have occasionally been isolated from lesions closely resembling those of Rio Grande gummosis, but have failed to induce gummosis upon inoculation. Olson and Waibel (13, 14) reproduced the Rio Grande gummosis syndrome in grapefruit in Texas by inoculations with *Diplodia natalensis*. In California, *Diplodia* species rarely have been found in lesions like those of Rio Grande gummosis. When *Hendersonula toruloidea* occurs in such lesions it is invariably associated with bacteria and other fungi. In Texas, *H. toruloidea* has not been found.

Recent experiments in California by the authors have shown that an unidentified fungus is capable of inducing a form of gummosis almost identical with the Rio Grande gummosis of Texas. In view of this development and the fact that *H. toruloidea* inoculations have caused only cankers or branch blight with associated gumming instead of Rio Grande gummosis, it may be assumed that *H. toruloidea* in lesions of the Rio Grande gummosis type is usually a secondary invader which begins its development on exposed necrotic tissue or on exudations from injuries.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BATES, G. R. 1937. Diseases of citrus fruits in Southern Rhodesia. Mazoe Citrus Exp. Sta. Pub. **6**: 173-208.
2. CALAVAN, E. C., AND J. M. WALLACE. 1948. Exosporina branch blight of grapefruit in southern California. (Abs.) Phytopathology **38**: 913.
3. CHILDS, J. F. L. 1953. An actinomycete associated with gummosis disease of grapefruit trees. Phytopathology **43**: 101-103.
4. FAWCETT, H. S. 1936. Citrus diseases and their control. McGraw-Hill Book Company, Inc., New York, N. Y. 656 p.
5. GODFREY, G. H. 1945. A gummosis of citrus associated with wood necrosis. Science **102**: 130.
6. GODFREY, G. H. 1946. Infectious wood necrosis and gummosis of citrus. Proc. Ann. Lower Rio Grande Valley Citrus Inst. **1**: 66-70.
7. KLOTZ, L. J. 1950. Gum diseases of citrus in California. Cal. Agr. Exp. Sta. Circ. **396**: 1-20.
8. KLOTZ, L. J. 1951. The battle against citrus diseases. Citrus Leaves **31**(8): 15, 38, 42.
9. MILBRATH, D. G. 1947. Branch wilt of walnut survey. Cal. Dept. Agr. Bul. **36**(4): 204-205.
10. NATTRASS, R. M. 1933. A new species of Hendersonula (H. toruloidea) on deciduous trees in Egypt. Brit. Mycol. Soc. Trans. **18**: 189-198.
11. NATTRASS, R. M. 1934. Annual report of the mycologist for the year 1933. Cyprus Dept. Agr. Ann. Rept. 1933: 48-57.
12. OLSON, E. O. 1952. Investigations of citrus rootstock diseases in Texas. Proc. Sixth Ann. Rio Grande Valley Hort. Inst. 1952: 28-34.
13. OLSON, E. O., AND C. W. WAIBEL. 1951. Studies on the transmission of Rio Grande gummosis of grapefruit. Proc. Fifth Ann. Rio Grande Valley Hort. Inst. 1951: 53-59.
14. OLSON, E. O., AND C. W. WAIBEL. 1953. Rio Grande gummosis in relation to Diplodia infection in Texas citrus. Proc. Seventh Ann. Rio Grande Valley Hort. Inst. 1953: 84-94.
15. PENZIG, O. 1887. Studi botanici sugli agrumi e sulli piante affini. Memoria Premiata dal R. Minister, dell' Agricoltura [Dagli Annali di Agricoltura 1887.] Tipografia Eredi Botta, Rome, Italy. 590 p.
16. WARNER, R. M. 1952. Some observations on branch wilt in figs. Proc. Sixth Ann. Res. Conf., Cal. Fig Inst. 1952: 24-25.
17. WILSON, E. E. 1945. A wilt disease of Persian walnut in California. U. S. Dept. Agr. Pl. Dis. Rptr. **29**: 614-615.
18. WILSON, E. E. 1947. The branch wilt of Persian walnut trees and its cause. Hilgardia **17**: 413-436.
19. WILSON, E. E. 1949. The pycnidial stage of the walnut branch wilt fungus, Exosporina fawcetti. Phytopathology **39**: 340-346.
20. WILSON, E. E. 1952. Factors affecting the branch wilt disease of walnuts. Proc. Sixth Ann. Res. Conf., Cal. Fig Inst. 1952: 21-23.

INHERITANCE OF RESISTANCE TO INFECTION BY TOBACCO-MOSAIC VIRUS IN TOMATO[1]

Francis O. Holmes

SUMMARY

Some tomato plants of hybrid origin have proved substantially more resistant to infection by tobacco-mosaic virus than others. Plants representing resistant lines were crossed with ordinary tomato varieties. A study was made of the segregation of resistance to infection in successive generations of progeny derived from these crosses. Results were compatible with the supposition that a single dominant gene controls an increased resistance to infection. True breeding resistant lines have not yet been obtained.

All edible varieties of tomato (*Lycopersicon esculentum* Mill.) are readily infected by tobacco-mosaic virus (*Marmor tabaci* H.). When infected, they yield a substantially smaller crop of fruit than healthy plants (1, 2, 3, 5). The wild Chilean tomato (*Lycopersicon chilense* Dun.) is not so readily infected, a characteristic known to be transmitted to some of the progeny of its hybrid with the edible tomato (6). The mode of inheritance of this characteristic was not determined in the earlier studies (6) because of the high degree of sterility in the hybrid lines. Subsequently, fruitful lines of tomato characterized by resistance to mosaic were obtained from Dr. W. A. Frazier.[2] When seedlings of these lines were inoculated with dilute suspensions of a typical mottling-type isolate of tobacco-mosaic virus, some plants proved to have a level of resistance to infection similar to that previously studied in derivatives of *Lycopersicon chilense*. The present paper reports the results of a study of these lines and their progenies, carried out with the purpose of determining the mode of inheritance of the tendency to escape infection by tobacco-mosaic virus.

Two levels of susceptibility were studied. The first of these was the level characteristic of all known named varieties of tomato. The second was the depressed level that permitted plants possessing it to escape infection, in most cases, when they were exposed to inoculations just sufficient to infect nearly all plants of ordinary tomato varieties. Plants characterized by the ordinary degree of susceptibility to infection will be referred to in this paper as susceptible and those characterized by the tendency to escape infection will be called resistant.

METHODS AND MATERIALS.—A strain of tobacco-mosaic virus, identified by the laboratory designation 1952D, was used for quantitative and, when necessary, repeated measurement of relative susceptibility of individual tomato seedlings. This strain of virus produces a conspicuous necrotic lesion at each point of its entrance and establishment both in leaves of tomato (8) and in leaves of tobacco (10). It was maintained in Turkish tobacco plants by single-lesion transfers. Juice expressed from these stock plants 9 to 12, but generally 10, days after infection was used to inoculate the tip leaflet of each of 3 leaves in testing tomato plants for relative susceptibility. Inoculated leaves of fully susceptible plants developed a substantial number of brown necrotic local lesions by the fifth day after inoculation (Fig. 1, A). Resistant plants usually showed no lesions at this time (Fig. 1, B); after a longer period they sometimes gradually developed a few relatively small necrotic lesions. Susceptible plants often permitted spread of virus to stems or to developing young leaves if the inoculated leaflets were allowed to remain attached to the plant indefinitely. Resistant plants did not. If plants were to be retested, all inoculated leaves were removed soon after counts of lesions had been recorded. This usually prevented movement of virus into stems of susceptible plants. Reinoculation with the same or some other strain of tobacco-mosaic virus then permitted confirmation of the degree of susceptibility previously recorded for each plant.

The mosaic-resistant lines of tomato used in this study were those described by Frazier and Dennett (4) and by Kikuta and Frazier (11). They had been developed in the Hawaiian Islands by selection for resistance to unidentified mosaic diseases in the field and by a later more rigorous selection to eliminate individuals mottling early after inoculation with a yellow-mottling strain of tobacco-mosaic virus. Knowledge of their original parentage is incomplete because they were derived from complex hybrids.

The resistant lines originally proved fertile in crosses with an ordinary susceptible variety of tomato (Rutgers) when used as female parents but not when used as male parents. After a few successive generations of backcrossing to the susceptible variety, however, fertility of resistant plants seemed about equal in reciprocal crosses.

MODE OF INHERITANCE OF RESISTANCE TO INFECTION.—Seedling progenies were derived from backcrosses of resistant to susceptible plants and from self-pollinations of resistant plants. These were inoculated with strain 1952D of tobacco-mosaic virus to determine ratios of resistant to susceptible plants in each progeny. Tests were made in successive generations over a period of 6 years.

Plants that proved as resistant to infection as the original resistant parent plants appeared in all prog-

[1] Accepted for publication June 17, 1954.

[2] Seeds of the mosaic-resistant tomatoes were supplied at the time Dr. Frazier was on the staff of the Hawaiian Agricultural Experiment Station.

FIG. 1. A) Terminal leaflet from a susceptible tomato seedling 5 days after inoculation with the 1952D strain of tobacco-mosaic virus. B) Corresponding leaflet from a resistant seedling similarly inoculated. Lesions produced in the susceptible leaflet were necrotic and dark brown in color; in some cases they were surrounded by blurred, chlorotic halos. The resistant leaflet showed no such lesions, although scratches produced mechanically by the process of inoculation appeared on it as well as on the susceptible control. (Photograph by J. A. Carlile.)

enies obtained from a series of crosses of resistant with susceptible lines. Thus it became evident that no recessive gene was of importance in conferring the resistance to infection. The number of seeds available for producing sets of backcross seedlings was usually small, especially in early tests, ranging from about 10 to 50 in each lot. In early generations, difficulty was experienced in obtaining germination even of such seeds as were available, but this trouble did not recur after a few generations. Fruit size increased in successive generations, as a result of backcrossing to large-fruited varieties of tomato. Plant size tended to be greater than in either parent in some of the crosses, presumably as an indication of hybrid vigor.

Backcrosses to susceptible varieties yielded approximately equal numbers of resistant and susceptible plants. In 7 progenies having susceptible varieties as pollen parents, the accumulated ratios amounted to 65 resistant to 68 susceptible plants. With reciprocal backcrosses, in which the pollen parents were resistant plants, accumulated ratios in 5 sets amounted to 52 resistant to 45 susceptible plants. These 2 series of backcross tests furnished a total of 117 resistant to 113 susceptible individuals. This approximate monohybrid ratio is regarded as evidence that only 1 segregating chromosome is concerned in the control of resistance to infection. The data seem fully compatible with the supposition that a single dominant gene determines this type of response to inoculation.

The tardy development of a few tiny necrotic lesions on inoculated leaves of some plants was puzzling for a time. These plants had been classed tentatively as resistant to infection. Usually no lesions had developed in their inoculated leaves within the time required for a full count of large lesions to appear on known susceptible plants. Consideration was given to the possibility that segregation of a second major gene, or of a number of minor genes, might have modified and weakened resistance in these plants. On this account an attempt was made to determine whether the plants that showed a few delayed, tiny lesions were inherently less resistant than plants that showed no lesions at all after initial inoculation. Ten plants that had produced 1–3 small necrotic lesions each as a result of the first inoculation were compared with 10 plants from the same set that had shown no lesions and with 9 additional plants that had proved fully susceptible by showing promptly a substantial number of large necrotic lesions. All these plants were reinoculated with a somewhat more infective sample of virus than had been used in the first test. The first set of 10 plants, which had produced an average of 1.6 small lesions initially, now developed an average of 3.3 small lesions. The second set of 10 plants, which had shown no lesions initially, developed an average of 3.4 small lesions. The 9 susceptible control plants, which had shown an average of 20.5 large lesions in the initial test, developed an average of 91.1 large lesions. Thus, in this test, no evidence was found that plants responding to inoculation by the delayed production of a small number of tiny lesions were any less resistant inherently than those that appeared to escape infection altogether in the initial test.

Self-pollination of resistant plants in the present study usually produced abundant seeds. These seeds, except in the earliest generations, germinated well and produced more seedlings than were available in corresponding backcross progenies. Ratios of resistant to susceptible plants were, however, lower than had been anticipated. In a series of 10 progenies derived by self-pollination of resistant plants, representing several successive generations, a total ratio of 450 resistant to 231 susceptible plants was recorded. This differed sharply from the expected monohybrid 1:2:1, or 3:1, ratio. It resembled rather a 0:2:1 ratio, which suggested that offspring bearing the gene for resistance in homozygous condition may never, or rarely, prove viable. No true-breeding resistant lines have been found.

Crosses have been made to a number of different tomato varieties. Progeny of these crosses have been examined closely to select individual plants bearing the desired characteristic of resistance to infection but closely resembling the susceptible parent in foliage characteristics and general habit. Selection of this kind has permitted the elimination from the resistant

lines of certain wild-type characteristics, especially a tendency to bushy growth as a result of excessive development of axillary shoots near the base of the plant. It is hoped that this selection procedure eventually may eliminate any recessive lethal factors that are linked with the gene for resistance and thus permit establishment of true-breeding resistant lines of tomato.

ORIGIN OF RESISTANCE.—Frazier's selected lines were derived from complex hybrids in which *L. chilense* and derivatives of this species had been included. Other wild species, such as *L. hirsutum* Humb. & Bonpl., *L. peruvianum* (L.) Mill., and *L. pimpinellifolium* Mill., had been involved also. It may or may not be possible eventually to determine which of these species contributed the gene for resistance to infection that now characterizes the selected lines.

The resistant tomato plants used in the present investigation seemed comparable in most respects to those previously derived from known crosses of *L. esculentum* and *L. chilense*. In the present study, resistance to infection is regarded as a dominant characteristic, whereas earlier investigations (6) had shown that the F_1 hybrid *L. esculentum* × *L. chilense* was intermediate in susceptibility as compared with its 2 parents. The distinction is of little value, however, because no plants bearing the gene for resistance to infection in homozygous condition were identified in the series of experiments based on Frazier's mosaic-resistant lines. Had such homozygous individuals been available for comparison, it is possible that the characteristic of resistance to infection might have appeared intermediate in dominance. Probably it will be necessary to await further evidence before reaching a conclusion on the question whether the types of resistance studied in the 2 series of experiments were identical.

In one respect the resistant tomato plants recently studied differed definitely from those originally derived by known hybridization with *L. chilense*. All tested resistant plants in the earlier experiments yielded inhibitory juices when crushed (6). It has not been possible to demonstrate similar inhibition of infection in *Nicotiana glutinosa* L. by mixing inoculum with juices expressed from the resistant plants used in the present investigation. This does not prove, however, that the types of resistance to infection were different in the 2 series of experiments. It was recognized earlier (6) that the inhibitory juice might be an incidental rather than an essential characteristic of resistant plants and that some plants characterized by resistance to infection might be found later not to yield inhibitory juices.

EFFECTIVENESS OF RESISTANCE TO INFECTION.—Resistant seedlings, and clones of plants derived by rooting stem cuttings from such seedlings, already have proved their value in experimental work. In an earlier publication (7) it was mentioned that circumstantial evidence connecting internal-browning disease of tomatoes with infection by certain strains of tobacco-mosaic virus was confirmed through the use of clones of the resistant tomatoes. The resistant lines escaped both mosaic mottling and internal browning of fruits in a heavily affected field. No other line of tomato had been found resistant to either of these. Other field trials have shown similar escape from infection with whatever strains of tobacco-mosaic virus have been present, or a substantial delay of infection after exposure.

On several occasions, cuttings from resistant plants that had been exposed to infection in diseased tomato fields throughout the summer were rooted and grown in a greenhouse during the following winter without becoming infected during summer exposure, autumn handling as cuttings, or winter maintenance. Susceptible varieties could not have been expected to remain healthy under such circumstances. The resistant tomatoes are by no means immune from infection, but their grade of resistance is a distinct asset in the presence of occasional and slight contaminative contacts.

THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, NEW YORK

LITERATURE CITED

1. ALEXANDER, L. J. 1950. Effect of tobacco mosaic virus on yield of glasshouse and field grown tomatoes. (Abs.) Phytopathology **40**: 1.
2. ALEXANDER, L. J. 1951. Effect of tobacco-mosaic disease on yield of tomato. (Abs.) Phytopathology **41**: 1.
3. ALEXANDER, L. J. 1952. Effect of the tobacco mosaic disease on the yield of unstaked tomatoes. (Abs.) Phytopathology **42**: 463.
4. FRAZIER, W. A., AND R. K. DENNETT. 1949. Tomato lines of Lycopersicon esculentum type resistant to tobacco mosaic virus. Proc. Am. Soc. Hort. Sci. **54**: 265-271.
5. HEUBERGER, J. W., AND A. J. MOYER. 1931. Influence of mosaic infection on tomato yields. Phytopathology **21**: 745-749.
6. HOLMES, F. O. 1943. A tendency to escape tobacco-mosaic disease in derivatives from a hybrid tomato. Phytopathology **33**: 691-697.
7. HOLMES, F. O. 1950. Internal-browning disease of tomato caused by strains of tobacco-mosaic virus from Plantago. Phytopathology **40**: 487-492.
8. HOLMES, F. O. 1952. Use of primary lesions to determine relative susceptibility of individual plants. (Abs.) Phytopathology **42**: 113.
9. HOLMES, F. O. 1952. Inheritance of resistance to tobacco-mosaic disease in tomato. (Abs.) Phytopathology **42**: 467.
10. HOLMES, F. O. 1953. Submutants from nonsystemic mutant strains of tobacco-mosaic virus. Indian Phytopath. **5** (1952): 8-16.
11. KIKUTA, K., AND W. A. FRAZIER. 1947. Preliminary report on breeding tomatoes for resistance to tobacco mosaic virus. Proc. Am. Soc. Hort. Sci. **49**: 256-262.

ISOLATION AND IDENTIFICATION OF L-PIPECOLIC ACID FROM WESTERN-X DISEASED PEACH LEAVES[1]

Theodor O. Diener and Charles A. Dekker[2]

SUMMARY

Paper chromatographic analysis of free amino acids in peach leaves affected with Western-X disease and systemic arsenic toxicity revealed an unidentified, ninhydrin-reacting substance which could not be detected in extracts prepared from mature, healthy leaves. The ninhydrin-reacting substance was isolated from Western-X diseased peach leaves by the use of column chromatographic methods and identified as L-pipecolic acid.

The effects of virus infection on the metabolism of stone fruit tissues are little understood. Rawlins and Thomas (9), investigating Buckskin disease, reported the accumulation of phenolic constituents in diseased peach leaves. O'Reilly (7), studying the physiological changes induced in peach foliage by the Western-X disease virus, found that diseased leaves respired more rapidly than healthy leaves, that foliar starch accumulation increased with disease severity, and that total nitrogen content was lower in diseased than in healthy foliage.

Investigations of virus-induced metabolic changes in stone fruit tissues were begun at the Irrigation Experiment Station in 1952, centering mainly on the free amino acid content of healthy and virus-infected leaf tissues. Western-X disease of peach was chosen for this study because it is known to affect, in its initial stages, only part of an infected tree (10), making it possible to collect control samples from unaffected portions of the diseased tree for comparison with those from healthy neighboring trees.

IDENTIFICATION OF FREE AMINO ACIDS IN PEACH LEAVES. — *Materials and methods.* — Samples from Western-X diseased peach trees were collected during the 1952 and 1953 seasons from the following counties in Washington: Benton, Yakima, Chelan, Douglas, Walla Walla, Kittitas, Stevens, and Grant. The represented varieties were: Elberta, J. H. Hale, Rochester, and Lovell seedlings. Fifty-three samples were obtained from diseased trees. Leaves from unaffected portions of the diseased trees and from apparently healthy neighboring trees were used as control samples.

The collected leaves were stored at −10°C. After thawing, 15 gm of leaves were extracted in an Omnimixer with 50 ml of 95 per cent ethanol at room temperature. The final ethanol concentration was approximately 80 per cent by volume. The extract was filtered and the filtrate vigorously shaken with 3 volumes of chloroform. The resulting water-rich layer was separated, and an aliquot of 10 ml was acidified with 2N hydrochloric acid (*ca* pH2) and added to 3 gm of Nalcite HCR cation exchange resin (20–50 mesh) in the H^+ cycle. After the sample had been mechanically shaken for 60 minutes, the supernatant was poured off and the resin was washed 8 times with distilled water. The amino acids were then eluted from the resin with 1 ml of 5N ammonium hydroxide, again shaken for 60 minutes and the supernatant used for paper chromatographic analysis. The amino acid solution was spotted on Whatman No. 1 paper (28.3 × 46.3 cm) with a micro pipette of 2 μl capacity: satisfactory chromatograms were obtained with 8 × 2 μl of extract.

The papers were developed in "Chromatocab" chambers (descending) using phenol-n-butanol-water (16–3–10, w/v/v) in the first and n-butanol-acetic acid-water (4–1–5, v/v/v) in the second dimension. After drying, the papers were sprayed with ninhydrin (0.1 gm dissolved in 95 ml of water-saturated n-butanol and 5 ml of collidine) and the color developed in an oven at 110°C. for 3 minutes.

Each sample of diseased leaves was processed, together with its corresponding control sample using identical extraction procedures, equal volumes of extract for the chromatographic analysis, and simultaneous chromatography in the same cabinet.

Results.—The following amino acids (and amides) could be identified by comparing their respective R_F values with those of pure compounds: aspartic acid, glutamic acid, serine, threonine, alanine, asparagine, glutamine, arginine, histidine, glycine, valine, tyrosine, trytophan, β-alanine, (iso)leucine, phenylalanine, γ-amino butyric acid and proline.

The consistent presence in Western-X diseased leaves of an unidentified, nihydrin-reacting substance and its apparent absence in mature healthy leaves was reported earlier (1). The substance gave a blue, rapidly fading spot with ninhydrin. Its R_F values were: 0.90 in water-saturated phenol, 0.32 in collidine-2,6-lutidine-water (1–1–1, v/v/v), and 0.34 in n-butanol-acetic acid-water. The color of the ninhydrin-treated spot in ultraviolet light was bright red in contrast to the dark blue observed with most other amino acids.

The chromatographic behavior of the unidentified substance strongly suggested its identity with pipecolic acid (pipecolinic acid, piperidine-2-carboxylic acid), an amino acid isolated from clover (6) and from beans (12), and which, apparently, is present in a large number of plant species (6).

Pure L-pipecolic acid and L-nipecotic acid (piperi-

[1] Accepted for publication June 23, 1954.
Scientific Paper No. 1319, Washington Agricultural Experiment Stations, Pullman, Washington, Project No. 590.
[2] Assistant Plant Pathologist, Irrigation Experiment Station, Prosser, Washington, and Assistant Professor of Biochemistry, University of California, Berkeley 4, California, respectively.

dine-3-carboxylic acid)[3] were compared chromatographically with the compound observed in extracts from diseased peach leaves. Addition of L-pipecolic acid to these extracts always resulted in a single spot, whereas addition of L-nipecotic acid resulted in 2 separate spots, L-nipecotic acid having a lower R_F value in both solvent systems used.

ISOLATION AND IDENTIFICATION OF L-PIPECOLIC ACID FROM WESTERN-X DISEASED PEACH LEAVES.—The extraction procedure described by Morrison (6) for clover was modified as follows: the leaves were dried at 70°C. for 12 hours and the dry material ground in a Raymond mill. After extraction of the ground leaves with 70 per cent ethanol, the filtered extract was evaporated to dryness, the residue taken up in water and the resulting sôlution extracted with chloroform. The aqueous layer was acidified to approximately pH2, filtered through Celite, and introduced onto a column of Dowex 50 cation exchange resin (50–100 mesh) previously converted to the free acid form. The amino acids were then eluted from the resin with 0.2N ammonium hydroxide and the amino acid solution evaporated to dryness under reduced pressure.

The residue was dissolved in 50 ml of a mixture of n-butanol, pyridine, and water (1–1–1, v/v/v) and 10 ml of the solution were introduced onto a column of Whatman cellulose powder (4 cm × 63 cm) previously equilibrated with the above solvent. The column was developed and the eluate collected in 10 ml fractions. The amino acid composition of each fraction was determined by one-dimensional paper chromatography, using water-saturated phenol as solvent. It was found to be as follows: (iso)leucine, fractions 14–18; valine, 17–25; pipecolic acid, 21–27; proline, 28–32; other amino acids, 32–78. Complete separation of pipecolic acid from all other amino acids except valine was, therefore, achieved. The procedure was repeated with the remaining 40 ml of the amino acid solution. All fractions containing pipecolic acid (and valine) were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of the lighter phase of a mixture of n-butanol, formic acid, and water (10–1–6, v/v/v) and introduced onto a column of Whatman cellulose powder (4 cm × 63 cm) previously equilibrated with the n-butanol-formic acid-water solvent. The distribution of amino acids in each fraction was again followed by means of paper chromatography. A complete separation of pipecolic acid from valine was achieved. The fractions containing pipecolic acid were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 50 ml of water and treated with 0.05 gm of charcoal. After filtration the solution was evaporated to dryness and the pipecolic acid repeatedly recrystallized from absolute ethanol-acetone. From 500 gm of dried leaves 76 mg of pipecolic acid were obtained in small, colorless needles, m.p. 268–269°C. (Morrison (6) gave m.p. 271°C.). After sublimation at 180°C./0.5 mm Hg pressure, the compound had a m.p. of 265°C. The specific optical rotation (measured in a 2 dm tube) was: $[\alpha]_D^{26}$ –26.1° in water (c, 1.12). The following analytical figures were obtained. Found: C, 55.6; H, 8.6; N, 10.6. Calculated for $C_6H_{11}O_2N$: C, 55.8; H, 8.5; N, 10.9 per cent. The isolated pipecolic acid was treated with potassium cyanate and the hydantoin was obtained as colorless needles, m.p. 136.5–137.5°C. (Leithe (4) reports m.p. 137–139°C.).

OBSERVATIONS ON THE OCCURRENCE OF L-PIPECOLIC ACID IN PEACH LEAVES.—Pipecolic acid could be detected in all 53 extracts from peach leaves showing symptoms of Western-X disease. The concentration of the amino acid, as judged by the color intensity and area of its corresponding spot on paper chromatograms, varied considerably. No consistent correlation between symptom severity and concentration of pipecolic acid could be established. Intense spots were sometimes obtained from extracts of leaves showing only slight symptoms of the disease and, conversely, in some cases weak spots were obtained from extracts of leaves with severe symptoms of Western-X disease.

With the method described no pipecolic acid could be detected in mature, healthy leaves. To determine whether, in mature, healthy leaves, pipecolic acid was present in combined form and, therefore, not extractable with ethanol, several samples of such leaves were hydrolyzed in 6N hydrochloric acid for 24 hours at 100°C. No pipecolic acid could be detected in the hydrolyzates, although pipecolic acid was known to resist the conditions of the hydrolysis.

However, when young, healthy peach leaves (1–2 cm long) were analyzed, a faint spot corresponding to pipecolic acid could generally be detected.

Symptoms somewhat resembling those caused by Western-X disease virus are observed with several disorders of peach trees, such as systemic arsenic toxicity (3) and nitrogen deficiency (8). Leaves from trees showing symptoms of these disorders were collected and tested for pipecolic acid. No pipecolic acid could be detected when leaves from girdled trees (wire or mouse damage) or leaves from trees showing symptoms of nitrogen deficiency were analyzed. When leaves from trees showing symptoms of systemic arsenic toxicity were analyzed, a spot corresponding to pipecolic acid was consistently found. The concentration of pipecolic acid in arsenic injured leaves appeared to be comparable to that in Western-X diseased leaves.

DISCUSSION.—The evidence secured made it reasonably certain that the isolated substance was pipecolic acid. However, the value obtained for the specific optical rotation was lower than that reported by either Mende (5) or Leithe (4) for synthetic L-pipecolic acid. The obtained value was comparable to that reported by Morrison (6) for pipecolic acid isolated from clover leaves. It appeared, therefore, that the preparation from Western-X diseased peach leaves was, like Morrison's preparation from clover, partially racemized.

[3] Supplied by Dr. G. Harris, Nutfield, Surrey.

The presence of pipecolic acid in Western-X diseased peach leaves leads to the consideration of its metabolic origin and its possible significance in virus infection. Pipecolic acid has been detected in a wide variety of plant species (6), where the amino acid appears to be a normal metabolite of the tissues involved. Grobbelaar and Steward (2) have shown that when ϵ-C^{14}-labeled lysine was introduced into bean plants the radioactivity was transferred to pipecolic acid. It appears, therefore, that in beans pipecolic acid is derived from lysine. A mechanism for such a conversion has recently been suggested (11).

The situation with peach leaves is different insofar as pipecolic acid does not accumulate in appreciable amounts in healthy leaves. Pipecolic acid might, therefore, be a break-down product of the injured host plant. However, since no consistent correlation between symptom severity (amount of necrosis) and concentration of pipecolic acid could be found and since traces of pipecolic acid were detected in young healthy leaves, this assumption seems to be unwarranted. The presence of small amounts of pipecolic acid in young, healthy leaves strongly suggests that the amino acid is a normal metabolite of peach leaf tissues which, however, only accumulates in appreciable amounts if the tissue suffers from certain disorders, such as Western-X disease or arsenic injury.

WASHINGTON STATE COLLEGE
IRRIGATION EXPERIMENT STATION
PROSSER, WASHINGTON
AND
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

LITERATURE CITED

1. DIENER, T. O. 1953. Presence of an unidentified ninhydrin-reacting substance in peach leaves affected with Western-X disease. (Abs.) Phytopathology **43**: 586.
2. GROBBELAAR, N., AND F. C. STEWARD. 1953. Pipecolic acid in Phaseolus vulgaris: Evidence on its derivation from lysine. Jour. Am. Chem. Soc. **75**: 4341-4343.
3. HARLEY, C. P. 1951. Arsenic injury. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Agr. Handb. 10, p. 250-251, illus.
4. LEITHE, W. 1932. Die Konfiguration des optisch aktiven Coniins und L-pipecolins. Ber. dtsch. chem. Ges. **65**: 927.
5. MENDE, F. 1896. Die Spaltung der Pipecolinsäure in ihre beiden optischen Componenten. Ber. dtsch. chem. Ges. **29**: 2887.
6. MORRISON, R. I. 1953. The isolation of L-pipecolinic acid from Trifolium repens. Biochem. Jour. **53**: 474-478.
7. O'REILLY, H. J. 1953. Physiological changes induced in peach foliage by the Western-X disease virus. (Abs.) Phytopathology **43**: 481.
8. PROEBSTING, E. L., AND O. W. DAVIDSON. 1951. Nitrogen deficiency. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Agr. Handb. 10, p. 222-223, illus.
9. RAWLINS, T. E., AND H. E. THOMAS. 1941. The buckskin disease of cherry and other stone fruits. Phytopathology **31**: 916-925.
10. REEVES, E. L., E. C. BLODGETT, T. B. LOTT, J. A. MILBRATH, B. L. RICHARDS, AND S. M. ZELLER. 1951. Western X-disease. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Agr. Handb. 10, p. 43-52, illus.
11. ROTHSTEIN, M., AND L. L. MILLER. 1954. Loss of the α-amino group in lysine metabolism to form pipecolic acid. Jour. Am. Chem. Soc. **76**: 1459.
12. ZACHARIUS, R. M., J. F. THOMPSON, AND F. C. STEWARD. 1952 . The detection, isolation and identification of (-)-pipecolic acid as a constituent of plants. Jour. Am. Chem. Soc. **74**: 2949.

CONTROL OF BACTERIAL WILT OF CHRYSANTHEMUMS WITH STREPTOMYCIN[1]

R. S. Robison, R. L. Starkey, and O. W. Davidson

SUMMARY

Chrysanthemum was found to be particularly well suited to studies of the effects of antibiotics on infection by *Erwinia chrysanthemi*, for it succumbed quickly and showed rapid response to antibiotics.

Results indicate that streptomycin and oxytetracycline can be used to control the bacterial wilt disease. The antibiotics were readily absorbed by and translocated in cuttings, but more slowly by rooted plants. They persisted, in amounts sufficient to provide protection against the disease, in plants that developed from the cuttings. Streptomycin retained activity in plants for several weeks. Streptomycin was only slightly toxic at the lowest concentration used and with some methods of treatment, but relatively toxic at high concentrations.

Streptomycin was applied by holding cuttings in solutions of the antibiotic, planting cuttings and rooted plants in antibiotic-treated sand, and treating cuttings with rooting hormone powder containing the antibiotic.

Streptomycin was more effective than oxytetracycline, for it provided protection at lower concentrations and was less toxic.

The potentialities of antibiotics for the control of plant diseases are becoming increasingly apparent as evidence accumulates on their inhibitive effects on various plant pathogens, their uptake by plants, their persistence in plant tissue, and their relative toxicity to the parasite and host (1, 7, 12, 14, 15, 16, 17, 18).

In recent tests, various antibiotics applied in different ways have been effective as plant chemotherapeutic agents. Streptomycin has been used as a spray to control halo blight of beans (19) and fire blight of apples (9), and as a dust for fire blight of pears (2). Cycloheximide has been used as a spray for the control of powdery mildew of roses (16) and as a dust to treat wheat seeds for covered smut (8). Peach trees were injected with oxytetracycline to control bacterial spot (5). Lettuce was protected from infection by *Botrytis cinerea*, and tomato plants from the early blight fungus by griseofulvin absorbed from the culture solution in which the plants were growing (3). Halo blight and common blight of beans have been controlled by antibiotics applied to stems in lanolin paste (13). Seed treatment with antibiotics also has proved effective in controlling certain bacterial and fungus diseases (12).

This report is concerned with the treatment of unrooted, as well as rooted, cuttings for control of the bacterial wilt of chrysanthemums caused by *Erwinia chrysanthemi* Burkholder et al (4). There appears to have been no previously reported use of antibiotics to treat unrooted cuttings for control of systemic diseases.

The bacterium produces a systemic infection. In unrooted cuttings the disease may involve: 1) complete destruction of the basal ends and wilting; 2) partial rotting of the base and absence of, or delayed, rooting; 3) no apparent destruction of the base or interference with root formation, but partial destruction of the vascular system. In the last case, plants develop for a time without evidence of disease, then suddenly wilt. At time of wilting, the pithy parenchymous tissue at the base and the roots near the soil surface have disintegrated. In young rooted cuttings the disease interferes with root development or results in destruction of the roots. In well developed plants there is wilting due to plugging of the vascular tissue, which becomes reddish-brown.

METHODS.—In an assay procedure for antibiotics in plant tissues, *Bacillus subtilis* Cohn *emend* Prazmowski (ATCC 6633) was used as the test organism. It was grown on nutrient agar of the following composition: beef extract 0.3 per cent, Difco peptone 0.5 per cent, agar 1.5 per cent. In cup tests, with the antibiotics in M/15 phosphate buffer at pH 6.5, the test culture was inhibited by solutions containing 1.0 p.p.m. or more of streptomycin or 0.4 p.p.m. or more of oxytetracycline. Presence of antibiotics in plants that had been treated with the substances was determined by a leaf disc method similar to a procedure of Leben and Keitt (11). A paper punch was used to obtain discs of leaf tissue 3.5 mm. in diameter. The discs were placed on solidified plates of nutrient agar seeded with *B. subtilis*, after which the plates were incubated for 24 hours at 28°C. Zones of inhibition about the discs served as evidence of presence of antibiotic in the tissues.

To determine the effects of leaf tissue on activity of the antibiotics, leaf discs and discs of filter paper[2] 3.5 mm. in diameter were immersed in solutions of streptomycin for 1 hour, then subjected to the agar-plate test. Inhibition by the discs of leaf tissue was similar to that of paper, but the zones from the leaf discs were somewhat smaller (Table 1). Zones around the discs varied considerably in size. Furthermore, when the period of exposure was shortened, the zones of the leaf discs were considerably smaller than those of the paper discs. The results indicate that a reasonable

[1] Accepted for publication July 3, 1954.

Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, The State University of New Jersey, New Brunswick, Departments of Agricultural Microbiology and of Horticulture. This project was supported in part by a grant from E. I. duPont de Nemours and Co. Chrysanthemum cuttings were generously provided by Yoder Brothers, Barberton, Ohio.

[2] Discs cut from Schleicher and Schuell analytical filter paper discs No. 740-E.

TABLE 1.—*Zones of inhibition produced about paper discs and leaf discs serving as carriers of streptomycin*

Antibiotic in test solution p.p.m.	Diameter of zones of inhibition, mm[a]	
	Paper discs	Leaf discs
0.5	7.0	6.9
1.0	9.6	7.5
5.0	15.7	13.8
7.5	16.6	16.5
10.0	17.8	16.8

[a] Averages of 8 or 9 replicates.

approximation of the antibiotic content of leaf tissue was obtained by the leaf disc method.

In most experiments the Indianapolis White variety of chrysanthemum was used. It was readily infected, and inoculated cuttings were quickly destroyed.

On streaked plates of nutrient agar, *E. chrysanthemi* was inhibited by 2.0 p.p.m. of streptomycin, 0.4 p.p.m. of oxytetracycline, 0.4 p.p.m. of chlorotetracycline, and 30 p.p.m. of neomycin.

EXPERIMENTAL RESULTS.—*Absorption, translocation, and persistence of antibiotics in plants.*—Unrooted cuttings were kept in distilled water containing 1000 p.p.m. of streptomycin or oxytetracycline. These substances are representative of 2 different types of antibiotics. Within 24 hours both antibiotics were detected in the upper leaves by the leaf disc method. Rooted cuttings growing in sand treated with 100 p.p.m. of streptomycin absorbed sufficient streptomycin to induce a chlorotic condition within 2 weeks.

Similar results were obtained in a commercial greenhouse,[3] where rooted cuttings were kept for 48 hours in Vermiculite wetted with a solution containing 150 p.p.m. of streptomycin, and then transplanted to soil. Though many leaves were chlorotic, the plants grew. Approximately 2 months after treatment some of the basal leaves were still chlorotic, but otherwise the plants appeared normal. Tests by the leaf disc method at this time showed presence of material having antibiotic activity in both the old bottom and new top leaves, but more commonly in the bottom leaves (Table 2). This may have particular significance in treatment of lower parts of plants which are not readily penetrated by certain other systemic materials. Not only had streptomycin been absorbed and translocated in the plants but appreciable amounts had persisted for more than 8 weeks.

In another experiment, cuttings were held for 4 hours in solutions containing 50, 150, 300, or 600 p.p.m. of oxytetracycline made up in M/30 phosphate buffer of pH 6.0, or for 6 hours in solutions containing the same amounts of streptomycin in distilled water. They were then rooted in sand for 21 days, transplanted to soil 36 days after the initial antibiotic treatment, and tested for activity. Twelve of 21 leaf specimens from the streptomycin series showed antibiotic activity, but only 3 of 19 from the oxytetracycline series.

[3] Burton O. Smith, Kennet Square, Penn.

TABLE 2.—*Antibiotic activity of leaves of streptomycin-treated plants*[a]

Chrysanthemum variety tested	Time elapsed between treatment and test, days	Antibiotic activity[b]	
		Top leaves	Bottom leaves
White Blanch	76	0/6	4/6
White Shasta	76	2/6	6/6
Reward	59	0/6	6/6
White Popcorn	59	2/6	2/6

[a] A solution containing 150 p.p.m. of streptomycin was added to the Vermiculite rooting substrate.

[b] Number of leaves showing antibiotic / number of leaves tested.

Guttation water appeared on leaves of certain cuttings that had been held in a solution containing 1000 p.p.m. of streptomycin for 12 hours. This water, as well as leaf discs from the plants, had antibiotic activity. The cuttings were rooted in sand and, when tested 14 days later, were found to contain active material. In some cases the zones of inhibition produced by the discs measured 28 mm. in diameter. These zones were greater than those produced by paper discs saturated with a solution containing 10 p.p.m. of streptomycin (Table 1).

Plant protection against wilt by antibiotics; inoculation after treatment.—The basal ends of cuttings were held in water solutions of streptomycin for 6 hours or in solutions of oxytetracycline in M/30 phosphate buffer at pH 6 for 4 hours, then washed, inoculated by dipping into a suspension of cells of the bacterium, dusted with a rooting hormone powder, and placed in sterilized sand in flats. There were also suitable controls.

Within 24 hours all inoculated cuttings that had not been treated with antibiotic solutions had succumbed to the disease (Table 3). Cuttings treated with the antibiotics showed varying degrees of protection against the disease and of injury from the antibiotics. Streptomycin was more effective than oxytetracycline in that it provided greater control against infection and was less toxic.

Evidence of toxicity was chlorosis, browning of leaf margins, inhibition of root formation, or inhibition of vegetative growth. Chlorosis appeared about the sixth day in basal leaves of cuttings treated with solutions containing 600 or 1000 p.p.m. of streptomycin. On the twenty-third day, many of the young apical leaves of cuttings treated with 50 or 150 p.p.m. of streptomycin were chlorotic. Apical growth did not appear to be inhibited, whereas cuttings treated with the more concentrated solutions of streptomycin were inhibited. Some basal leaves of cuttings treated with 300 to 1000 p.p.m. of streptomycin were chlorotic or had brown margins, and some apical leaves became almost white and many died.

Treatment with 600 or 1000 p.p.m. of oxytetracycline produced initial wilting, from which the cuttings recovered slowly. All the cuttings treated with oxytetracycline showed chlorosis by the fourteenth day.

TABLE 3.—*Influence of antibiotic treatment on persistence of cuttings inoculated with E. chrysanthemi*[a]

Treatment	Antibiotic p.p.m.	Number of cuttings surviving[b]	Root development
Noninoculated controls	0	15	15 very good
Inoculated controls	0	0	
Streptomycin	50	15	11 good, 4 fair
Streptomycin	150	15	13 fair to good, 2 poor
Streptomycin	300	15	6 poor, 5 very poor, 4 none
Streptomycin	600	15	8 poor, 2 very poor, 5 none
Streptomycin	1000	6[c]	6 none, 9 rotted
Oxytetracycline	50	13	9 fair to poor, 4 very poor, 2 diseased
Oxytetracycline	150	14[c]	4 poor, 9 very poor, 1 none, 1 diseased
Oxytetracycline	300	14[c]	7 very poor, 7 none, 1 diseased
Oxytetracycline	600	10[c]	2 very poor, 8 none, 5 rotted
Oxytetracycline	1000	9[c]	9 none, 6 rotted

[a] Period of development in sand: for streptomycin 25 days, for oxytetracycline 22 days.
[b] Fifteen cuttings for each treatment.
[c] Antibiotic was toxic.

Chlorosis was general throughout the leaf, which became yellow-green, the color being nearly the same in cuttings treated with solutions having low and high concentrations of the antibiotic. In contrast, bleaching due to streptomycin appeared first along the vascular system, progressed outward toward the leaf margins, and in severe cases caused the leaf to become nearly white.

Toxicity of both antibiotics at the highest concentrations was evident as rotting of basal ends of the cuttings, presumably by saprophytes. Root development was better the lower the concentration of antibiotic used, and it was better with streptomycin than with oxytetracycline. None of the cuttings treated with streptomycin succumbed to the disease, whereas several of those treated with oxytetracycline became diseased.

Cuttings treated with 1000 p.p.m. of streptomycin failed to produce roots. This has been noted repeatedly. It is nevertheless possible to obtain good root development of cuttings treated with much lower concentrations of streptomycin, which provided protection against disease.

Disease control by antibiotics; inoculation before treatment.—Cuttings were inoculated by dipping the basal ends in a suspension of cells of the pathogen, after which they were kept in tap water for 6 hours to favor bacterial penetration. As a result of infection, the basal ends showed slight browning and varying degrees of softness. The cuttings were washed in water and placed in solutions of the antibiotics as in previous tests. They were held in the streptomycin solutions for 6 hours and in the oxytetracycline solutions for 5 hours. The cuttings that had been treated with streptomycin were turgid, whereas those treated with oxytetracycline were somewhat flaccid. The basal ends were treated with rooting hormone and planted in sand.

Within 24 hours all inoculated cuttings that had not been treated with antibiotic had succumbed to the disease. Six cuttings treated with oxytetracycline at 75 p.p.m. were lost from disease in 8 days, but none of those treated with streptomycin was thus lost. The basal ends of all cuttings treated with antibiotics turned black from effects of the pathogen, but the rot did not progress beyond this stage in the antibiotic-treated cuttings. Chlorosis appeared on the cuttings treated with oxytetracycline in 8 days and on those treated with 800 p.p.m. of streptomycin in 13 days. The condition of the cuttings after 20 days is indicated by Table 4. The results indicate that both antibiotics provided protection against disease but that streptomycin gave better protection and was less toxic than oxytetracycline. Root development on cuttings treated with antibiotics under the conditions prevailing was inferior to that on untreated cuttings. The reduced root development can be attributed in part to the initial infection of the basal ends of the cuttings.

In another experiment, cuttings were inoculated by dipping the basal ends into a suspension of the patho-

TABLE 4.—*Control of bacterial wilt of chrysanthemums by antibiotic treatment*[a]

Treatment	Antibiotic p.p.m.	Number of cuttings surviving out of 15 treated	Number of cuttings rooted	Root development
Noninoculated controls	0	15	15	15 good
Inoculated controls	0	0	0	
Streptomycin	75	15	14	8 good, 4 fair, 2 very poor
Streptomycin	225	15	5	5 very poor
Streptomycin	450	15	9	1 fair, 5 poor, 3 very poor
Streptomycin	800	15	6	2 poor, 4 very poor
Oxytetracycline	75	9	8	4 fair, 3 poor, 1 very poor
Oxytetracycline	225	15	7	7 very poor
Oxytetracycline	450	15	7	7 very poor
Oxytetracycline	800	15	2	2 very poor

[a] Period of development in sand 20 days.

TABLE 5.—*Treatment of rooting sand with streptomycin for prevention of bacterial wilt of chrysanthemums*[a]

Treatment	Antibiotic p.p.m.	Number of cuttings surviving out of 10 treated	Root development
Noninoculated controls			
Inoculated controls	0	10	10 good
Streptomycin	0	0	.....
Streptomycin	50	9	9 good
Streptomycin	150	9	8 good, 1 fair
Streptomycin	450	9	4 good, 5 fair
Streptomycin	800	10	1 good, 3 fair, 3 poor, 3 none

[a] Period of development in sand 16 days.

gen, as in the preceding experiment, but the cuttings were immediately planted in sterilized sand that had been treated with streptomycin. Antibiotic treatment consisted of adding solutions containing 50, 150, 450, or 800 p.p.m. of the antibiotic to sand in 7-in. pots until liquid drained from the bottom. The surface 2 inches was thoroughly mixed and then compacted before the cuttings were planted.

All inoculated cuttings that lacked antibiotic treatment succumbed within 48 hours, whereas almost all the cuttings treated with streptomycin survived. Root development was poor on cuttings kept in sand treated with the 2 largest amounts of streptomycin (Table 5).

The basal ends of the cuttings in sand that had been treated with the smallest amount of streptomycin disintegrated, but the rot did not progress. The amount of basal rot decreased with increase in the amount of antibiotic used. Similar results were obtained in other experiments. Treatment with streptomycin had not only a protective action, but served to control an established infection. None of the protected cuttings became chlorotic.

In a similar experiment, in which the effects of both streptomycin and oxytetracycline were tested, both antibiotics afforded protection. Streptomycin had unfavorable effects on root formation at the higher concentrations, but oxytetracycline was inhibitive at all levels used.

The use of antibiotic-treated sand should be an effective means of preventing spread of the wilt disease among cuttings in rooting beds and should prevent development of the disease in cuttings that become inoculated while being prepared for rooting.

Treatment with antibiotics in rooting hormone powder.—The basal ends of cuttings were dipped into a cell suspension of the pathogen, after which they were dipped into rooting powder containing 0.1 per cent indole-butyric acid in talc and either 1 per cent or 10 per cent streptomycin. Some of the excess powder was removed by tapping the cuttings, but more remained attached than the normal amount. The cuttings were planted in sterilized sand.

All the inoculated controls succumbed to the infection within 4 days, whereas all those treated with streptomycin survived (Table 6). The cuttings treated with streptomycin failed to root properly, however. Those treated with the powder containing 1 per cent streptomycin showed delayed root development due to destruction of basal tissue by the pathogen; the basal 1-in. portions of the stems had disintegrated before progress of the infection stopped. Lack of root development on cuttings receiving powder with 10 per cent streptomycin is ascribed to toxicity of the antibiotic. It is possible that lower concentrations than those used would have provided protection. The auxin may have increased the toxicity of the antibiotic (10), but also it may have increased the inhibitive effect of the antibiotic on the wilt bacterium (6).

Whereas this method of treatment controlled the disease, it gave less satisfactory results under the prevailing conditions than did rooting of the cuttings in sand to which streptomycin had been added.

Persistence and vigor of plants produced from treated cuttings.—Plants that developed from cuttings held in solutions of streptomycin or oxytetracycline for absorption of antibiotic and then inoculated were grown in soil and observed for evidences of delayed development of the wilt disease and toxic effects of the antibiotics. These were some of the plants of a previously discussed experiment (Table 3). The results in Table 7 were obtained 94 days after the cuttings were treated with the antibiotics and 72 days after the plants were transferred from the rooting sand to soil. Similar results were obtained with cuttings that were infected and then treated with antibiotics.

If it is assumed that the loss of plants at the lowest level of the antibiotic was due to infection, and that loss at the higher dilutions was due to toxicity of the antibiotic, streptomycin afforded better protection and was less toxic than oxytetracycline. Nearly all the plants at the 2 lower levels of streptomycin survived. Streptomycin at higher concentrations delayed bud

TABLE 6.—*Prevention of bacterial wilt of chrysanthemums by treatment of cuttings with rooting powder containing streptomycin*

Treatment	Antibiotic in powder per cent	Number of cuttings surviving out of 18 treated	Number of cuttings rooted	Root development
Noninoculated controls	0	18	18	18 good
Inoculated controls	0	0	0	.....
Streptomycin	1	18	10	7 fair, 3 poor
Streptomycin	10	18	0	.....

TABLE 7.—*Development of plants from cuttings treated with antibiotics and then inoculated with E. chrysanthemi*[a]

Treatment	Antibiotic p.p.m.	Number of cuttings rooted and transplanted out of 15 treated	Number of plants surviving	Number of plants with buds
Noninoculated controls				
Inoculated	0	15	14[b]	12
controls	0	0	0	—
Streptomycin	50	15	13	12
Streptomycin	150	15	13[b]	4
Streptomycin	300	8	5	2
Oxytetracycline	50	13	11	5
Oxytetracycline	150	13	3	0
Oxytetracycline	300	7	1	0

[a] Condition of plants 94 days after treatment.
[b] One cutting lost in handling.

formation, but oxytetracycline had greater inhibitive effects.

Somewhat better results were obtained with the plants that developed from cuttings inoculated and then rooted in sand treated with streptomycin. These are the cuttings referred to in Table 5. After being kept in sand for 16 days for rooting, the cuttings were planted in soil. Eighty-six days after treatment and 70 days after being planted in soil the plants showed no evidence of toxicity at 50 and 150 p.p.m. of streptomycin, slight toxicity at 450 p.p.m., and appreciable toxic effects (severe chlorosis) only at 800 p.p.m. Almost complete protection against infection was obtained with all concentrations of the antibiotic. Buds were formed on all plants, but there was somewhat poorer bud formation on the plants treated with the higher concentrations of streptomycin.

FIG. 1. Control of chrysanthemum wilt by streptomycin. Left to right: plants from inoculated cuttings treated with 50 p.p.m., plants from non-inoculated untreated controls, plants from inoculated cuttings treated with 800 p.p.m.

At later stages of growth there were no gross differences between the treated and untreated plants, as shown by Figure 1. The plants bloomed, were pinched back, and had bloomed the second time when the photograph was made, 105 days after treatment.

DEPARTMENTS OF AGRICULTURAL MICROBIOLOGY
AND HORTICULTURE
AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

1. ANDERSON, H. W., AND D. GOTTLIEB. 1952. Plant disease control with antibiotics. Econ. Bot. **6**: 294-308.
2. ARK, P. A. 1953. Use of streptomycin dust to control fire blight. U. S. Dept. Agr. Pl. Dis. Reptr. **37**: 404-406.
3. BRIAN, P. W., J. M. WRIGHT, J. STUBBS, AND A. M. WAY. 1951. Uptake of antibiotic metabolites of soil microorganisms by plants. Nature **167**: 347-349.
4. BURKHOLDER, W. H., L. A. MCFADDEN, AND A. W. DIMOCK. 1953. A bacterial blight of chrysanthemums. Phytopathology **43**: 522-526.
5. DUNEGAN, J. C., R. A. WILSON, AND W. T. MORRIS. 1953. Effects of terramycin on peach trees affected with bacterial spot. U. S. Dept. Agr. Pl. Dis. Reptr. **37**: 604-605.
6. GOODMAN, R. N., AND D. D. HEMPHILL. 1954. The effects of indole-3-acetic acid on the plant disease-inhibiting properties of antibiotics. Science **119**: 347-348.
7. GREGORY, K. F., O. N. ALLEN, A. J. RIKER, AND W. H. PETERSON. 1952. Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa. Phytopathology **42**: 613-622.
8. HENRY, A. W., R. L. MILLAR, AND E. A. PETERSON. 1952. Control of covered smut of wheat by rapid seed treatment with an antibiotic. Science **115**: 90-91.
9. HEUBERGER, J. W., AND P. L. POULOS. 1953. Control of fire blight and frog-eye leaf spot (black rot) diseases of apples in Delaware in 1952. U. S. Dept. Agr. Pl. Dis. Reptr. **37**: 81-83.
10. IYENGAR, M. R. S., AND R. L. STARKEY. 1953. Synergism and antagonism of auxin by antibiotics. Science **118**: 357-358.
11. LEBEN, C., AND G. W. KEITT. 1949. Laboratory and greenhouse studies of antimycin preparations as protectant fungicides. Phytopathology **39**: 529-540.
12. LEBEN, C., AND G. W. KEITT. 1954. Effects of antibiotics in control of plant diseases. Jour. Agr. and Food Chem. **2**: 234-239.
13. MITCHELL, J. W., W. J. ZAUMEYER, AND W. H. PRESTON, JR. 1954. Absorption and translocation of streptomycin by bean plants and its effect on the halo and common blight organisms. Phytopathology **44**: 25-30.
14. NICKELL, L. G., AND A. C. FINLAY. 1954. Antibiotics and their effects on plant growth. Jour. Agr. and Food Chem. **2**: 178-182.
15. PRAMER, D. 1953. Observations on the uptake and translocation of five actinomycete antibiotics by cucumber seedlings. Ann. Appl. Biol. **40**: 617-622.
16. VAUGHN, J. R., J. L. LOCKWOOD, G. S. RANDWA, AND C. HAMNER. 1949. The action of actidione on plant tissue and upon certain fungi. Mich. Agr. Exp. Sta., Quart. Bul. **31**: 456-464.
17. WEINDLING, R., H. KATZNELSON, AND H. P. BEALE. 1950. Antibiosis in relation to plant diseases. Ann. Rev. Microbiol. **4**: 247-260.
18. WRIGHT, J. M. 1951. Phytotoxic effects of some antibiotics. Ann. Bot., n.s. **15**: 493-499.
19. ZAUMEYER, W. J., H. R. THOMAS, J. W. MITCHELL, AND H. H. FISHER. 1953. Field control of halo blight of beans with streptomycin. Phytopathology **43**: 407.

A STUDY OF CANE AND CROWN GALLS ON VANCOUVER ISLAND AND A COMPARISON OF THE CAUSAL ORGANISMS[1]

W. E. McKeen[2]

SUMMARY

On Vancouver Island, crown and cane galls are very common on cane-fruit plants to the extent that every plant on some Boysenberry and Himalaya-blackberry plantations is infected with cane-gall-producing bacteria. The cane-gall organism, *Agrobacterium rubi* (Hildebrand) Starr & Weiss 1943, is systemic in its host and usually causes galls to form at nodes, but sometimes induces elongated ridges of tumorous tissue to appear on internodes. Virulent gall-producing bacteria have been isolated from healthy-appearing regions in floral canes. Cane galls have never been observed occurring naturally on primo canes but readily develop after inoculation.

The 8 gall-producing isolates studied were separated into 6 strains by physiological reactions, host range, and nutritional requirements. As more isolates were obtained, it became more difficult to differentiate the crown-gall organism (*Agrobacterium tumefaciens* (E. F. Smith & Towns.) Conn., 1942) from *A. rubi*. Isolates 2 and 4, which were obtained from the Himalaya blackberry, were quite distinct. All isolates were attenuated in glycine media.

Surveys during the summer of 1952 on Vancouver Island showed that galls were present in abundance not only on cane-fruit plants, but also on roses and other ornamental plants; galls appeared to be much more prevalent and serious in this area than in other parts of Canada. The prevalence of the disease has been due in part to the large number of susceptible hosts grown in this region and to the fact that new plantations have been started frequently in contaminated soil and in close proximity to diseased hosts.

One or 2 original plantings of each variety which apparently suffered from the disease have served as propagating stock for the majority of cane-fruit growers, thus carrying the disease from plantation to plantation. This practice has continued because of the lack of a propagating nursery, and because of the fact that healthy-appearing tips of Loganberry, Boysenberry, and Himalaya-blackberry may be carrying bacteria which cause cane gall. The importance of galls was frequently underestimated. In many instances, they were not observed on the parent stock.

Although the crown-gall disease has frequently been described and the cane-gall disease has been studied by Banfield (1), Hildebrand (4), and Coleman (3), no critical comparison has been made of these diseases or of the causal organisms. For this reason and because of the wide distribution and importance of the gall diseases on Vancouver Island, this study was undertaken.

ECONOMIC IMPORTANCE.—It is very difficult to estimate accurately the damage caused by galls, because the plants are not killed outright and, in some cases, seem to thrive without apparent damage, especially if they have been infected for only a short time. Thus it is impossible to generalize as to the kind and extent of injury which may result. The amount of injury varies with the host, its age at time of infection, locations of the galls, number of infections, and type of host response. However, there is usually a general unthriftiness and dwarfing in the affected plants, the leaves are smaller and dry earlier in the season, and the fruit is small, dry, and of poor quality and flavor. The galls, especially on the crowns and roots, serve as avenues of entrance for rot-producing fungi and bacteria. Cane galls usually do not endanger the life of the plant, since the floral canes are pruned off at the end of the season, but as they frequently split the canes (Fig. 4), the part distal to the gall usually dies after splitting occurs.

The decline in Loganberry and Boysenberry plantations and the reduction in yield has been due in part to this disease. Although Himalaya blackberries are seriously infected, sometimes to the extent of 100 per cent of the plants in a plantation, they usually do not appear to be injured to any great extent, presumably due to their extremely vigorous growth.

SYMPTOMS.—The galls on plants of *Rubus* spp. are of 2 kinds: crown galls caused by *Agrobacterium tumefaciens* (E. F. Smith & Towns.) Conn, 1942, and cane galls caused by *A. rubi* (Hildebrand) Starr & Weiss, 1943. The crown galls are restricted to the crown and root region of the plant, whereas the cane galls may develop on the roots, crowns, or floral canes.

A. tumefaciens may produce galls up to 12 in. in diameter on the crown, and smaller galls on the roots (Fig. 11), but not on the aerial parts of the plant. The gall tissue dies and partially disintegrates each fall and winter and a new gall is formed during the next growing season (Fig. 10). Galls are usually formed slightly below the ground level and frequently are unnoticed by the grower. If they are produced slightly above the ground, they are always dark in color, in contrast to the white galls that form beneath the soil, and they break down much more rapidly.

The cane-gall organism, *A. rubi*, produces small spherical growths or elongated ridges of white granular tissue (Fig. 2) on the floral canes during May and June. Galls usually appear during late April or early May on the stumps of the old fruiting canes (Fig. 8 and 9), and a few days later occur almost simultan-

[1] Accepted for publication June 24, 1954.
Contribution No. 1382 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Associate Plant Pathologist.

1
2
3
4
5
6
7
8
9
10
11
12

eously all along the cane which sometimes reaches a length of 20 ft. in Himalaya-blackberry plants. Predominantly, the galls occur at the nodes (Fig. 3 and 5), but sometimes elongated ridges of white tissue develop at any region. Galls invariably form on injured tissue. They rapidly increase in size, sometimes becoming 1 in. in diameter within a week; they may completely cover sections of the cane (Fig. 1). Enlargement of the galls may cause the cane to split, after which the terminal portion commences to dry and die. Galls appear on the roots, especially if they have been injured. The same syndrome is presented on Loganberry, Himalaya-blackberry, Boysenberry, and Youngberry plants.

The blueberry plant responds to the causal organism in a slightly different manner from that of plants belonging to the Rosaceae. Numerous small intumescences appear around the stem and usually along it for some distance (Fig. 6).

HOST-PARASITE RELATIONS.—The gall-producing bacteria are wound parasites and gain entrance into the hosts through injuries to the roots, pruning wounds, or other injuries. Little is known about the movement of bacteria within the cane-fruit plants, but such movement must occur, because the galls that develop predominantly at the nodes could hardly be the result of separate infections. In order to test this possibility, isolations were made from regions of fruiting canes of Himalaya blackberry in which no galls were present, and cultures of pathogenic organisms were obtained. This indicates that the disease is systemic in this host, and only 1 wound infection is necessary for the production of several widely separated galls on the cane.

Galls have never been found on primo canes. This may be due to freedom from bacteria or the inability of virulent bacteria to produce the necessary stimulus for excessive cell development. The bacteria probably either remain during the winter in the crown and roots and move into the year-old canes in early spring, or are always present in the young canes. Due to the fact that primo canes from the Himalaya blackberry can be used for propagation by tips when they are 2 months old, the author was able to obtain young plants from these very young canes and put them in a disease-free area to determine whether the young canes contain virulent bacteria despite the absence of galls.

The severity of cane gall on systemically infected plants fluctuates to some degree from season to season in Himalaya-blackberry plantations; this fluctuation may be due to the varying degrees of winter injury that occur. During the spring of 1950, galls developed in great profusion when the average temperatures in January and February were 10 and 6°F. lower, respectively, than for the same months of the other years from 1948 to 1952 in which gall production was light.

STUDIES ON THE GALL-PRODUCING BACTERIA.—Eight isolates of gall-producing bacteria were used in the investigation and a ninth was isolated recently and used in some experiments. The isolates were obtained from various plants and scientists in widely separated places (Table 1). Purity of cultures was assured by using single colonies from dilution plates. Thermal death points were determined by immersing, for 10 min., small test tubes (1cm. in diam.), which contained very young active cultures in yeast-extract broth (pH 7), in a liter of water maintained at a constant temperature.

Slices of carrot tissue were cut from healthy carrots aseptically and placed in a small amount of sterile water in petri dishes before they were inoculated. In some instances, roots grew from the tumorous tissue as they sometimes do under natural conditions (Fig. 7 and 12). All the isolates have been attenuated after 25 transfers in the glycine medium which van Lanen *et al* (7) used for the attenuation of crown-gall bacteria. However, the author added yeast extract to the medium in order that the bacteria requiring vitamins might grow.

The oxygen consumption of isolates 1 and 2 was measured at various pH values by means of the Warburg constant volume respirometer, and it was found that the oxygen consumption paralleled fairly closely the growth rate on agar media at pH values from 4 to 9. However, the respiration rate of isolate 2 was at least twice as great as that of isolate 1 at comparable hydrogen ion concentrations.

Since tomato and *Vicia faba* were used as differential hosts, they are the only ones mentioned in Table 1. However, for example, sunflower, Himalaya blackberry, raspberry, Boysenberry, salmonberry, sugar beet, sweet pea, garden pea, egg plant, common bean, pepper, and zinnia were susceptible to isolates 4 and 5.

Table 1 gives the results of pathogenicity, physiological reactions, and effect of temperature on the isolates. The data show that there is a wide range of variation and that the bacteria cannot be readily separated into 2 species.

←

FIG. 1–12. Fig. 1. The end of a Himalaya-blackberry floral cane, which is almost completely covered with tumorous tissue.—Fig. 2. A section of a Himalaya blackberry cane, which has several elongated ridges of tumorous tissue.—Fig. 3 A gall which surrounds the stump of a lateral branch on a Himalaya-blackberry cane.—Fig. 4. A portion of a Boysenberry cane, which has been split by elongated tumorous tissue.—Fig. 5. A portion of a Boysenberry cane, which has galls at each side.—Fig. 6. A portion of a blueberry stem, which has numerous small intumescences extending completely around and along it for some distance.—Fig. 7. A gall on the crown of a chrysanthemum plant. Observe the roots which have emerged from the gall.—Fig. 8. The base of a floral cane and the stump of an old cane surrounded by tumorous tissue. Galls on the stumps usually appear just prior to their appearance on floral canes.—Fig. 9. A young plant which has grown from the tip of a cane. There is a gall on the stump of the old cane, but there are no galls on the young plant.—Fig. 10. A young white gall growing over an old black gall on a Loganberry plant.—Fig. 11. A Loganberry crown with tumorous tissue on its crown and on a root. Very little normal tissue remains in the crown.—Fig. 12. Roots growing from small galls on a carrot slice which was inoculated with isolate 7.

TABLE 1.—*A comparison of pathogenicity, physiological reactions, and effect of temperature on 9 gall-producing isolates*

Isolate No.	1	2	3	4	5	6	7	8	9
Source of isolate	from A. J. Riker via L. C. Coleman	Himalaya-blackberry cane	crown of chrysanthemum	Himalaya-blackberry root	crown of red raspberry	*Achillea ptarmica* from P. A. Ark	Loganberry root	*Euonymous* from A. C. Braun	Boysenberry cane
Galls produced on tomato	+	−	+	+	+	+	+	+	−
Galls produced on *Vicia faba*	−	+	+	+	+	+	+	+	+
Tissues affected in carrot slices	phloem	cambium, xylem	cambium, phloem	cambium, phloem	cambium, phloem	cambium, phloem	xylem, phloem, cambium	phloem	
Vitamin requirements	−	+	−	+	+	−	−	+	
Color reaction in litmus milk	blue	pink	blue	pink	pink	blue	no change	blue	blue
Color reaction in bromocresol purple 1% glucose in beef broth	purple	faded	purple, blue ring at top	yellow	yellow	slightly pink, blue ring at top	yellow	purple	
Color reaction in phenol red and nutrient broth	red	yellow	red	yellow	yellow	red	red	yellow	
Reaction in nutrient broth	flakes, pellicle	turbid	flakes, pellicle	turbid	turbid	flakes, pellicle	turbid, few flakes at top	turbid	
Thermal death point in °C.	51–52	51–52	51–52	48–49	48–49	51–52	52–53	50–51	
Maximum growth temperature in °C.	40	32	40	32	32	40	34	40	
Oxygen consumption	1	2							

DISCUSSION.—As more isolates of the gall-producing *Agrobacterium* species were obtained and studied, the list of differential characteristics used by Hildebrand (4) for separating the cane and crown-gall organism proved more unsatisfactory. Physiological and nutritional tests and host-range studies have shown that the range of variation is very great. The author studied 6 distinct strains, and many more probably exist. Isolates 2 and 4 were obtained from the same host and locality, and were quite distinct. However, isolates 3 and 6 were obtained from different hosts and widely separated areas, and were quite similar.

Isolates 4 and 5 turned litmus milk very pink, a characteristic of *A. rubi*, but their host range included many non-*Rubus* plants and was even greater than that reported by Coleman (3) for his isolate from the Himalaya-blackberry plant.

Starr (6) indicated that the nutritional requirements of members of the genus *Agrobacterium* might be used in classifying the species. The 2 cultures of *rubi* he examined required calcium pantothenate, biotin, nicotinic acid, and dl glutamic acid or casein hydrolyzate and the cultures of *tumefaciens* did not require vitamins. However, although isolates may have the same nutritional requirements, their host range and other characteristics may be quite different. Also, Braun (2) has shown that Eu 7 only requires 3 vitamins not including dl glutamic acid or casein hydrolyzate and has a very wide host range. Because of this, he suggests that it might be considered a new species or strain of the crown-gall bacterium which has lost its ability to synthesize all the vitamins essential for optimum growth. It would appear, therefore, that there is no reliable list of differential characteristics for the separation of *A. tumefaciens* and *A. rubi*.

Klein and Klein (5) were able to change the host range of an isolate of *A. rubi*, which was similar to the author's isolate 2, so that tomato was a new host and thus its host range became similar to that of isolates 4 and 5. They postulated that specific host virulence is controlled by multiple genetic factors. If this be true, isolate 4 might change to isolate 2 by mutation and vice-versa under natural conditions.

Regardless of the relationship of gall-producing *Agrobacterium* species, certain ones produce galls on the floral canes, crown, and roots of cane-fruit plants, whereas others produce galls only on the crown and roots. Since the crown-gall organism readily produces galls on inoculated canes, it would seem that an explanation for the absence of galls on the canes might be the inability of this organism to move up the canes.

The reason for lack of galls on primo canes has not been ascertained, but there are at least 2 possible explanations: the pathogenic bacteria may not enter the young canes during the growing season, or the bacteria in the canes may be able to initiate gall development only when growth is beginning in the spring.

PLANT PATHOLOGY LABORATORY
SAANICHTON, B. C.
CANADA

LITERATURE CITED

1. BANFIELD, W. M. 1930. Cane gall of black raspberry. Phytopathology **20**: 123-124.
2. BRAUN, A. C. 1950. A tumor-inducing bacterium isolated from Euonymous galls. Phytopathology **40**: 1058-1060.
3. COLEMAN, L. C. 1950. Tumor induction in Vicia faba and other hosts by Agrobacterium rubi (Hildebrand) Starr and Weiss. Can. Jour. Res. C. **28**: 277-282.
4. HILDEBRAND, E. M. 1940. Cane gall of brambles caused by Phytomonas rubi n. sp. Jour. Agr. Res. **61**: 685-696.
5. KLEIN, DIANA T., AND R. M. KLEIN. 1953. Transmittance of tumor-inducing ability to avirulent crown gall and related bacteria. Jour. Bact. **66**: 220-228.
6. STARR, M. P. 1946. The nutrition of phytopathogenic bacteria. II—The genus Agrobacterium. Jour. Bact. **52**: 187-194.
7. VAN LANEN, J. M., J. L. BALDWIN, AND A. J. RIKER. 1952. Attenuation of crown-gall bacteria by cultivation in media containing glycine. Jour. Bact. **63**: 715-722.

THE RESPONSE OF TEN CLONAL LINES OF BROMUS INERMIS TO INFECTION BY SELENOPHOMA BROMIGENA [1]

Hugh Wintersteen

SUMMARY

Ten selected clonal lines of *Bromus inermis* were observed in a smooth brome grass nursery to determine the prevalence of *Selenophoma* leaf spot disease. Observations covered 2 growing seasons. During the first year's observations only 3 clonal lines, all located in 1 replication, were found to be infected, 1 lightly and 2 severely. Similar results were observed the second year except that the lightly infected clone of the previous year did not show any signs of infection.

The same 10 clonal lines were used in inoculation studies in the greenhouse. Data resulting from this study indicated that significant differences existed among these 10 clones with respect to resistance to infection by *S. bromigena* under greenhouse conditions.

From the results, 5 of the clones were arbitrarily classified as susceptible and 5 as resistant. The clones classified as resistant may prove of value in future smooth brome grass breeding experiments.

Within the past 20 years *Bromus inermis* Leyss has become an important forage grass in Iowa and now occupies an estimated 1,000,000 acres. Reports in the literature indicate that infection by *Selenophoma bromigena* (Sacc.) Sprague & A. G. Johnson has occasionally resulted in severe crop losses. Since resistant varieties seem to offer the only feasible method of control, a study was undertaken to evaluate the relative resistance of 10 agronomically desirable clonal lines.

Allison (1) made a detailed study of the disease as it occurred under Minnesota conditions, and Sprague and Johnson (3) reported that *Selenophoma* leaf spot was in part responsible for reducing the acreage of smooth brome grass in the Dakotas. In Iowa, Welch (5) reported striking differences of infection among clonal lines maintained in nursery plots, while Tsiang (4) found that highly significant differences in reaction to *Selenophoma* leaf spot occurred among clonal lines in the field.

MATERIALS AND METHODS.—The 10 clonal lines were maintained in a polycross nursery consisting of 72 entries replicated 6 times in a randomized block design and located at the Agronomy Farm, Ames, Iowa. Readings of the incidence of infection of *Selenophoma* leaf spot were taken during the spring and summer of 1952 and 1953.

In the fall of 1952, 20 plants of each of the 10 clonal lines were established in 6-in. pots in the greenhouse, and during the winter these 10 clonal lines were subjected to 8 inoculation trials.

The same strain of the pathogen which was isolated from the leaf tissues collected in the field was used for inoculum increase purposes. Copious sporulation was induced using the following medium and method. Five gm. of wheat meal were added to 30 cc. of 2 per cent potato dextrose agar in a 250 Erlenmeyer flask and the flask was then autoclaved for 20 minutes. After cooling, the flask was seeded with a spore suspension and stored for 2 weeks. The surface of the medium in the flask would then be covered with a bright pink mucoid type of growth. Although few well-defined pycnidia were visible, many partially hidden ones must have been formed as abundant cirri protruded above the surface.

Spore suspensions were made from these cultures and atomized onto the host leaves. The treated plants were placed in a moist chamber for 48 hours and then returned to the greenhouse bench.

Relative resistance of the clonal lines was evaluated in the following manner. Two weeks after inoculation, or later if disease expression was retarded, leaves of each plant were cut off and counted. The leaves were then individually examined to determine which ones showed symptoms of infection and count was made of the infected leaves. One lesion on a leaf was enough

[1] Accepted for publication July 27, 1954.
Journal Paper No. J-2529 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1178.

TABLE 1.—*Percentage of infected leaves of Bromus inermis resulting from inoculations of Selenophoma bromigena onto selected clones in the greenhouse, with corresponding angles in degrees*

Clonal designation	Mean percentage	Mean angle	Plants showing only flecking response
19	81	67	0
8	59	51	1
471	48	44	0
53	41	39	4
42	35	36	0
254	33	33	0
262	25	27	4
623	23	27	5
567	15	21	6
510	12	18	2

to cause that leaf to be classified as infected. A percentage-of-infected leaves value was then computed.

EXPERIMENTAL RESULTS.—Infection under field conditions was limited: only 3 clonal lines located in 1 replication showed disease symptoms, the other 5 replications being disease free both years. In 1952 clones 19 and 254 were heavily infected and clone 42 was lightly infected, while in 1953 clones 19 and 254 were again heavily infected.

Results of 8 inoculations in the greenhouse on different sets of these clonal lines are in Table 1. On some plants all infection was of a flecking nature. Clonal lines 510, 567, 623, 262, and 53 consistently showed this type of symptom expression and because of this fact and also their disease-free condition in the field, they were classified as resistant.

Since the data were numerical and not of measurement type and since the counts of diseased leaves were less than 100 in every case, the infection percentage figures were transposed into their corresponding angles to reduce the possibility of dealing with an aberrant distribution (2).

Analysis of variance indicated that there were highly significant differences among these 10 clonal lines with respect to percentage-of-leaves infected by *S. bromigena.*

DISCUSSION.—The exact nature of the relative resistance of the clonal lines to infection by *S. bromigena* was not determined. In both years that field observations were made, spread of the disease was limited to 2 or 3 clones all located in 1 replication. Almost 100 per cent of the leaves of clones 19 and 254 were infected and leaf blighting and severe leaf chlorosis were common symptoms. Differences among infected clones and non-infected clones were striking and absolute. Welch (5) reported similar observations.

In the greenhouse a different condition prevailed in that all lines showed some susceptibility and the clones differed as much in type of expression as they did in degree of expression. Clones 510, 567, 623, 262, and 53 consistently showed only flecking or relatively small lesions as a response to infection whereas the other clones on occasion were severely damaged by large coalescing lesions.

Under field conditions and considering a wide range of plants and plant diseases, the most vigorous plants are commonly the most severely parasitized. Clone 19, which proved to be the most susceptible of the 10 clonal lines tested, was a vigorous grower in the field and was the most vigorous growing of all the lines while growing under greenhouse conditions.

Clone 254 produced an abundance of succulent leaf material in the field and was severely infected. Early in the winter its growth was limited and the percentage of leaves infected under these conditions was relatively low. However, as the days became longer and greenhouse temperatures increased due to the sunny afternoons, plants of this clonal line showed a marked increase in growth rate and also a decided increase of percentage-of-leaves infected.

Clone 262, in contrast, produced a minimum of stooling in the field and in the greenhouse, and the sparse growth produced was harsh to the touch. This clone showed no infection in the field and whereas fungus penetration was effected after inoculations in the greenhouse, lesions which did develop were always few in number and restricted in size.

The association between growth types of clones 19, 254, and 262 and their response to infection was taken as an indication that vigor and susceptibility of *Selenophoma* leaf spot possibly were related.

BOTANY AND PLANT PATHOLOGY SECTION
IOWA AGRICULTURAL EXPERIMENT STATION
AMES, IOWA

LITERATURE CITED

1. ALLISON, J. L. 1945. Selenophoma bromigena leaf spot on Bromus inermis. Phytopathology **35:** 233-240.
2. SNEDECOR, G. W. 1946. Statistical Methods. Ed. 4. Ames, Iowa. The Iowa State College Press. 485 p.
3. SPRAGUE, R., AND A. G. JOHNSON. 1950. Species of *Selenophoma* on North American grasses. Corvallis, Oregon, College Press.
4. TSIANG, Y. S. 1944. Variation and inheritance of certain characters of brome grass, *Bromus inermis* Leyss. Jour. Amer. Soc. Agron. **36:** 508-522.
5. WELCH, A. 1945. Unpublished annual report. Ames, Iowa, Dept. of Bot. and Pl. Path., Iowa State College.

HOST PATHOGEN RELATIONS IN POWDERY MILDEW OF BARLEY
1. HISTOLOGY OF TISSUE REACTIONS[1]

N. H. White and E. P. Baker[2]

SUMMARY

Histological observations were made on whole leaves of varieties of barley and other cereals inoculated with *Erysiphe graminis* var. *hordei*, Marchal, when cleared and stained in hot lacto-phenol-cotton blue solution. Germination of conidia and formation of appressoria was the same on all hosts examined. On oats, rye, and wheat, either no penetration occurred or further development of the pathogen was arrested at the papilla stage, thus confirming the findings of earlier workers. An exception was the wheat variety Federation, which behaved similarly to highly resistant varieties of barley. On susceptible, semi-resistant, resistant, and highly resistant varieties of barley, penetration was effected and normal haustoria were formed. Mycelial development appeared to be related to the number of haustoria formed, and this in turn appeared to be limited by mesophyll collapse. It is claimed that resistance in barley to infection by *E. graminis hordei* is a function of the rapidity and degree of mesophyll collapse rather than being the result of death of epidermal cells or aborted development of haustoria.

Previous histological studies on tissue reactions of susceptible, resistant, and immune hosts to infection by species of the *Erysiphaceae* were confined to changes occurring in the infection court. Using freehand or microtome sections the workers paid special attention to reactions of infected cells of the host and to haustorial development by the pathogen. Their findings may be summarized as follows: development of powdery mildew on resistant and immune hosts is arrested before haustoria are formed at the papilla stage (4); no sac or disorganizing membrane is formed around haustoria on resistant hosts as on hosts resistant to rusts (5); and physiological resistance manifests itself in some hosts by death of infected epidermal cells (3, 8) and in others by distorted or aborted development of haustoria (3, 4).

That histological observations should be extended to host tissues other than those of the infection court is indicated by the work of Allen and Goddard (1, 2) who reported greatly increased respiration of mesophyll tissue beneath the infection court in wheat infected with powdery mildew. In the work reported in this paper, histological observations were made on entire infected leaves and on the pathogen after clearing and staining in hot lacto-phenol cotton blue solution. This technique has many advantages over sectioning with its attendant limitations. It permits overall observations and reveals details of both pathogen and host as well. Killed or severely injured host cells stain deeply in contrast to cells that are living when the leaves are immersed in the clearing and staining solution. The efficacy of this technique in this regard may be readily verified by artificially injuring or killing the cells (Fig. 1, A).

Preliminary observations revealed first that barley varieties considered immune on the basis of macroscopic appearance were in fact infected by the pathogen with the formation of normal haustoria in the epidermal cells. Secondly, in these and resistant varieties of barley the mesophyll cells had collapsed beneath the infection courts (Fig. 1, B) whereas in susceptible varieties there was no mesophyll collapse.

MATERIALS AND METHODS.—*Erysiphe graminis* var. *hordei* Marchal was the pathogen used and the inoculum was the same as that used at Sydney University for genetical work on the reactions of barley to powdery mildew. On Cherewick's (3) differential set it resembled his race 3. The barley varieties gave 4 types of macroscopic reactions, namely, susceptible, semi-resistant, resistant, and highly resistant (formerly thought to be immune) corresponding to the reaction types of Mains and Dietz (6), types 4, 3, 1 and 0 (with very slight flecking) respectively. The unrelated cereal hosts were Algerian oats, cereal rye, Federation (W 107)[3] and Kenya (W 744) varieties of wheat.

Seven day old seedlings were inoculated by dusting them with conidia from susceptible barley hosts. The inoculated plants were kept in the glasshouse and specimens for examination were taken at intervals as required by cutting off the top 2 in. of the first leaves. The specimens were immediately immersed in about 10 cc of lacto-phenol containing 1 per cent cotton blue and then brought to boiling over a low bunsen flame and allowed to simmer for 3 minutes, care being taken not to boil too hard. The tissue was kept in this solution until cold, though no harmful effect arose from keeping it in this solution overnight; the leaves were removed and gently rinsed in water and then mounted in clear lacto-phenol solution on a glass slide and covered with $2 \times \frac{7}{8} \times 0$ cover slips. The preparations were examined first under the binocular dissecting microscope at a magnification of 40 ×, and then for detailed study under the low and high power objectives of the compound microscope. Although all preparations were examined within a day after clear-

[1] Accepted for publication June 24, 1954.

The writers gratefully acknowledge the help given to them by the Department of Illustration for the preparation of the text figures.

[2] Senior lecturer in Plant Pathology and lecturer in Genetics and Plant Breeding respectively, in the Faculty of Agriculture, University of Sydney, Australia.

[3] W and B numbers refer to number in Sydney University varietal accession lists.

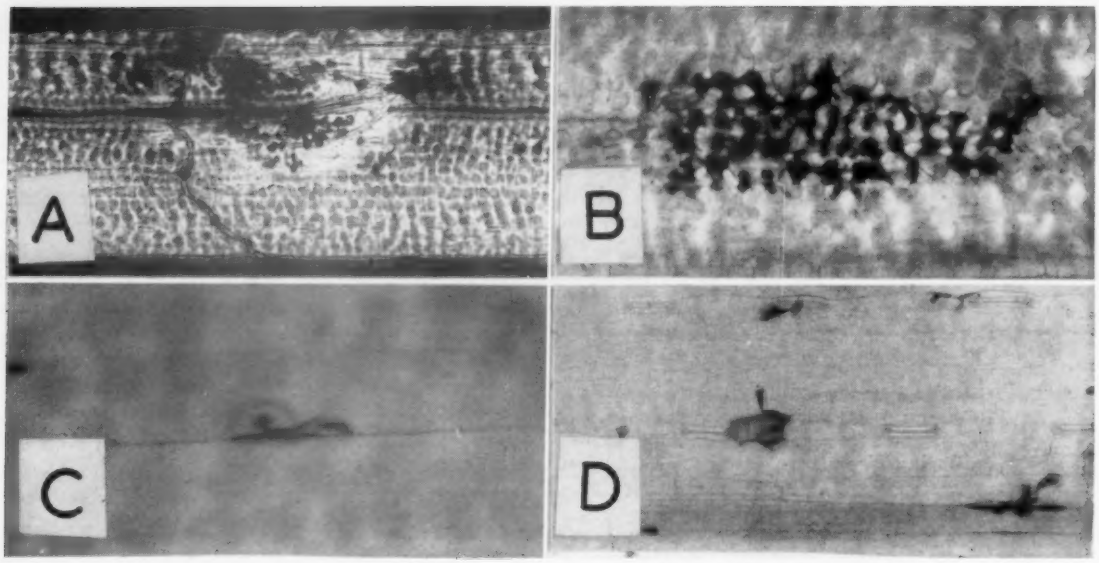


FIG. 1. A) Needle puncture on barley leaf of susceptible variety showing deeply staining dead and injured cells (× 75). B) Collapsed mesophyll of highly resistant variety of barley beneath infection court (× 150). C) The halo around the point of penetration by *E. graminis hordei* (× 300). D) Collapsed infected epidermal cell on highly resistant barley (× 150).

ing and staining, it was found they could be kept without deterioration for at least 6 weeks. Under the dissecting microscope both mycelial and conidial development of the pathogen, and the state of the host tissue could be seen. Under the higher powers of the microscope, by lowering the objective through the different optical planes, details of both host and pathogen were seen in the cleared tissue.

Although the haustoria do not stain so deeply as the remainder of the fungal tissue, their digitate form makes them readily distinguishable. Quantitative observations were attempted. To do this for each host variety, 20 colonies derived from single conidia were observed and counts made of the number of haustoria per infection court, the number of cells in the longest hyphal branch from conidium to tip, the number of collapsed mesophyll cells below the infection court, and the number of collapsed epidermal cells.

RESULTS.—*Histological observations on barley varieties 7 days after inoculation with E. graminis var. hordei.*—Varieties giving the 4 types of macroscopic reactions were used.

a) *Susceptible* (B 49, Kinver).—There was abundant development of mycelium and conidia on the leaf surface. There were 20–30 haustoria in the epidermal cells beneath each colony. The mesophyll appeared normal and collapsed cells were absent. An occasional epidermal cell had collapsed (Fig. 2, A).

b) *Semi Resistant* (B 28, Purple nudum C.1.2250 and B 69, No. 22).—In macroscopic appearance these varieties were obviously infected but each infection site was surrounded by a zone of dead host tissue. Histologically the mycelium was well developed in each colony, but was less dense than in the susceptible host. Conidial development was sparse compared with that on the susceptible host. There were 10–15 haustoria in the epidermal cells beneath each colony. Large groups of mesophyll cells had collapsed beneath and surrounding each colony. In some of the infection courts 1–2 epidermal cells had collapsed (Fig. 2, B).

c) *Resistant* (B155, Portuguese, B165, Portuguese and B167, Goldfoil).—The mycelium was restricted in development, giving the colonies a thin appearance, and a few conidial branches were produced by each colony. There were 5–12 haustoria in the epidermal cells beneath each colony. Small groups of mesophyll cells had collapsed beneath or near the infection court, and 2–5 epidermal cells had collapsed in many of the infection courts (Fig. 2, C).

d) *Highly Resistant* (B56, *Hordeum spontaneum* and B278, Triple bearded lemma. C.1.6766).—From macroscopic appearance these were formerly considered to be immune, but from microscopic study were re-classified as being highly resistant. Mycelial development was very limited and sparse. Occasionally 1 conidial branch was formed by a colony. Each infection court contained usually 1 but sometimes 2 haustoria in the epidermal cells. Beneath each infection court the mesophyll cells had collapsed. In many infection courts at least 1 epidermal cell had collapsed (Fig. 2, D).

Observations on cereals other than barley 7 days after inoculation with E. graminis var. hordei.

a) *Algerian Oats.*—There was no observable macroscopic symptom. Short germ tubes and appressoria were formed from the conidia and in most a penetration papilla was developed, but there was no further development of the pathogen, nor was there any evidence of host reaction.

b) *Rye.*—Again there were no macroscopic symp-

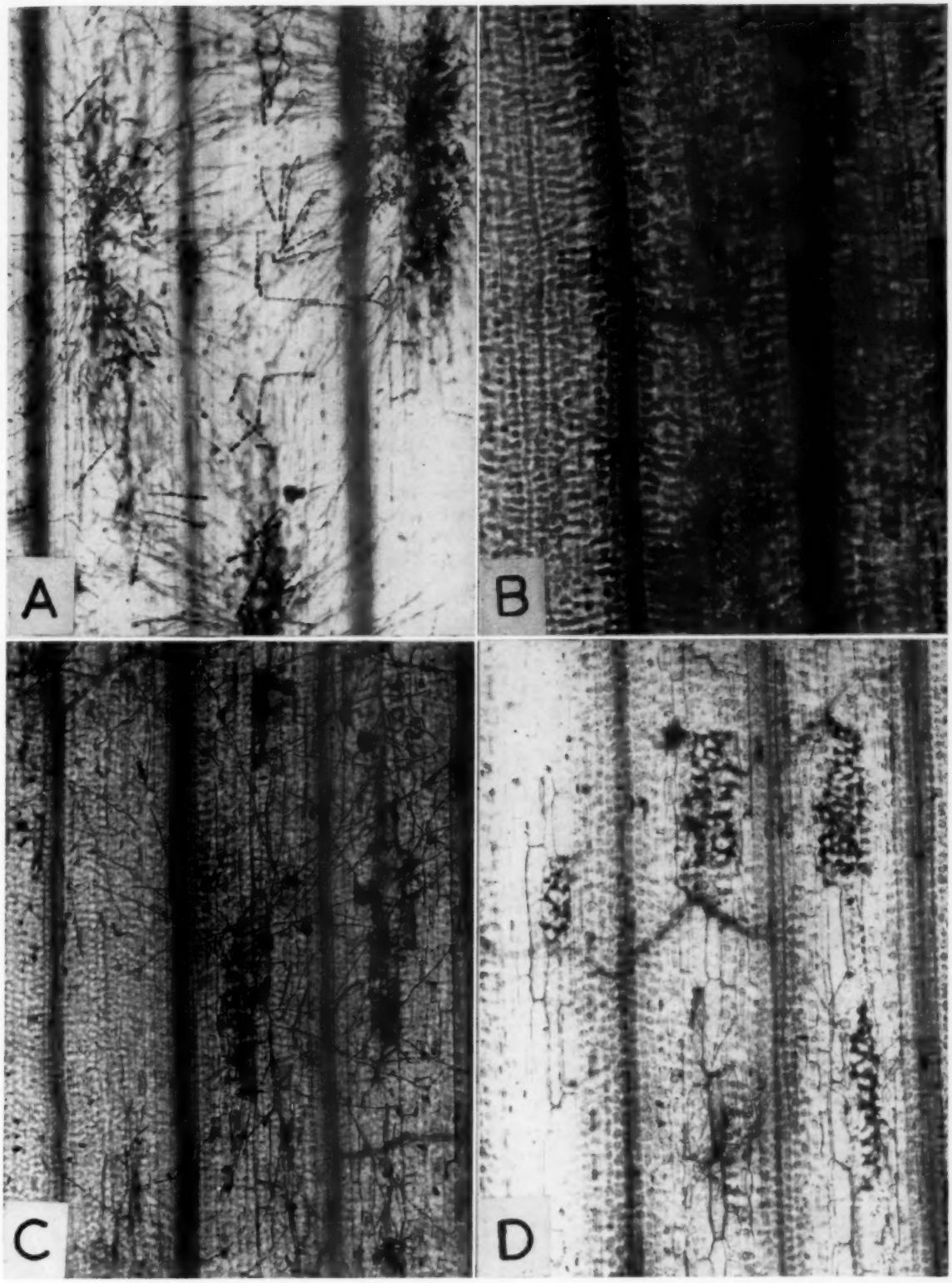


FIG. 2. Microscopic appearance of barley varieties reacting differently 7 days after inoculation with *E. graminis hordei* after being cleared and stained in hot lacto-phenol cotton blue (× 75). A) Susceptible. B) Semi-resistant. C) Resistant. D) Highly resistant.

toms. Histlogical examination showed only germ tube and appressorial development with no penetration papillae formation. Occasionally an epidermal cell had collapsed beneath the germ tube.

c) *Federation Wheat.*—Although no definite macroscopic symptom was shown, histologically there was infection of the host with a limited development of mycelium and 1 or 2 haustoria in the epidermal cells of the infection court. Beneath each infection court the mesophyll cells had collapsed, and in some the epidermal cells had collapsed. The reactions of this variety of wheat resembled closely those of the highly resistant barley varieties.

d) *Kenya Wheat* (W744).—There were no macroscopic symptoms but short germ tubes and appresoria were formed. There was no infection, nor any host reaction.

Histological observations on barley varieties giving the 4 types of reactions during the 4 days after inoculation with E. graminis var. hordei.—The results of observations made on barley varieties 7 days after inoculation suggested that the reactions of the hosts to infection and early development of the pathogen might be important in determining the ultimate relationship of the barley host to this pathogen. Samplings were therefore made at intervals during the first four days after inoculation and the results of these observations are summarized in Table 1.

It was found that germination of conidia, formation of appressoria, and penetration of the host was the same on all 4 types of barley and that infection was accomplished during the first 24 hours under glasshouse conditions. In all a typical halo in stained tissue surrounded the infection peg (Fig. 1, C).

Within 30 hours after inoculation an haustorium was formed in each infection court on all types of barley. In the highly resistant varieties fewer haustoria were developed in the infection courts. At this stage in these hosts where an haustorium had formed, 2–5 mesophyll cells collapsed immediately beneath the cell containing the haustorium. Sometimes an epidermal cell collapsed after infection, but the haustorium was formed in an adjoining living epidermal cell. (Fig. 1, D).

It will be seen from Table 1 that the amount of growth of mycelium and the number of haustoria

TABLE 1.—*Summary of the histological observations on barley hosts during the first 4 days after inoculation with E. graminis hordei. Mean and range of 20 observations.*

Mycelial Development	Hours after inoculation	Susceptible varieties	Semi-resistant varieties	Resistant varieties	Highly resistant varieties
Number of cells per longest hypha	6	Germ tube	Germ tube	Germ tube	Germ tube
	24	Infection	Infection	Infection	Infection
	30	2	2	2	2
	48	5	5	5	4
	54	6	6	6	5
	72	10	10	7	5
	96	18[a]	14	10	5
Number of haustoria per infection court	6	0	0	0	0
	24	odd one	odd one	few	rare
	30	1	1	1	few
	48	3	3	3	1
	54	3	3	3	1–2
	72	6	6	4	1–2
	96	12	10	5	1–2
Number of collapsed mesophyll cells per colony of the mildew	6	0	0	0	0
	24	0	0	0	1[b]–3
	30	0	0	0	2 –5
	48	0	few–side	0	6 –7
	54	0	3–4[c]	0	6 –8
	72	0	5–10	4–7	8 –10
	96	0	25–30[d]	6–8	10 –18
Number of collapsed epidermal cells in the infection court	6	0	0	0	0
	24	0	0	0	Some
	30	0	0	0	0–1[f]
	48	0	0	Rarely	0–1[f]
	54	0	0	Rarely	0–1[f]
	72	0	few	Some[e]	0–2[g]
	96	0	few	Some	0–3[h]

[a] Conidial branches formed at this stage.
[b] Immediately beneath infected cell containing the haustorium.
[c] To the side of the infection court.
[d] Surrounding and beneath the infection court.
[e] One cell in about 30 per cent of the infection courts.
[f] One collapsed epidermal cell in 50 per cent of infection courts.
[g] One to 2 collapsed epidermal cells in 70 per cent of infection courts.
[h] Two to 3 collapsed epidermal cells in 70 per cent of infection courts.

formed per infection court was the same during the first 54 hours for susceptible, semi-resistant, and resistant barley hosts. It is significant that there was no collapse of mesophyll cells during that time.

In the susceptible host there was unrestricted development of haustoria and mycelium during the first 4 days after inoculation and there was no collapse of the mesophyll or epidermal cells.

In the highly resistant hosts, mesophyll collapse began when an haustorium was formed within 30–48 hours after inoculation. Rarely were 2 haustoria formed in the infection court once host cell penetration was effected. The haustoria in these hosts were no different in size and shape from those formed in the susceptible host. Further mycelial development of the pathogen was arrested 54 hours after inoculation, when 6–8 mesophyll cells had collapsed beneath the infection court. Mesophyll cells collapse continued during the next 2 days, when there was no more growth of the pathogen.

In the resistant hosts, mesophyll collapse did not begin until 54–72 hours after inoculation and until this time haustorial and mycelial development kept pace with that on the susceptible host. From the time of mesophyll collapse the development of haustoria and mycelia were restricted, so that by the end of 4 days after inoculation there was only an average of 5 haustoria in an infection court compared with 12 in a susceptible host. There was correspondingly less development of the mycelium.

In the semi-resistant hosts, mesophyll collapse began around, but not beneath, the infection courts 48 hours after inoculation. There was no lessening in the development of haustoria and mycelium, when compared with the susceptible host, until between the third and fourth day after inoculation. At this stage there was a rapid collapse of mesophyll cells beneath and around the infection courts, involving 25–35 cells. With this mesophyll collapse there was the restriction in development of haustoria and mesophyll as seen in the resistant hosts.

Discussion.—From the evidence presented it is clear that normal haustoria are formed in all varieties of barley giving susceptible, semi-resistant, resistant, and highly resistant ("immune") reactions to infection by *Erysiphe graminis* var. *hordei*. The successful establishment of the pathogen, measured by the amount of mycelial and conidial development, is directly related to the number of haustoria formed by the colonizing pathogen in an infection court. The number of haustoria appears to depend on the rapidity and degree of mesophyll collapse beneath the infection court.

In susceptible barley there is no mesophyll collapse and the mildew attains its maximum development. In the varieties formerly considered immune but now reclassified as being highly resistant, because infection occurs on them, mesophyll collapse begins as soon as the single haustorium is formed in the infected epidermal cell but never before the haustorium is formed. On these hosts there is minimum development of the pathogen. In resistant barley, mesophyll collapse is delayed and occurs only after 3 or 4 haustoria are formed. Development of the pathogen is restricted only after the collapse of the mesophyll takes place. In semi-resistant hosts, there is a similar sequence of events except that extensive mesophyll collapse is delayed until 6–10 haustoria are formed by the colony and then large blocks of mesophyll cells collapse, limiting further development of the mildew. There is sufficient mycelial development to enable the formation of a number of conidial chains. Old infections can be seen macroscopically and are surrounded by necrotic tissue.

On cereal hosts other than barley the reactions are in agreement with those recorded by previous workers (3, 4). Penetration of the epidermal cells either does not occur or is not followed by formation of haustoria. These hosts are immune from *E. graminis* var *hordei*. One (Federation wheat) behaved similarly to highly resistant barley varieties and should therefore be considered as being highly resistant.

Resistance in barley to *E. graminis* var. *hordei* appears then to depend mainly on the sensitivity of mesophyll cells, reacting necrogenically to the presence of haustoria in epidermal cells, rather than either to death of epidermal cells (3, 8), or to aborted or distorted development of the haustoria (3, 4), as claimed by previous workers. Cherewick (3) observed that "in plants showing resistance marked by necrosis not only were the infected cells of the epidermis gradually killed but also the adjacent mesophyll cells." However, he did not emphasize the importance of collapse of the mesophyll in giving resistance to the host. The part played by collapse of infected and surrounding epidermal cells appears to be minor, but cannot be entirely ignored. There was a decreasing amount of epidermal collapse as there was a decrease in resistance from highly resistant to susceptible.

The concept of super-sensitivity of host cells as a basis for resistance due to necrogenic defense reactions was first elaborated by Müller in his work on host-pathogen relations of *Phytophthora infestans* on potato (7). In this work the speed of the necrogenic defence reaction confering resistance was considered the decisive factor. The more sensitive the cells, the more speedily they were killed by the presence of the pathogen. Müller[4] has suggested a 2-way action for these necrogenic defence reactions. First, substances are released from infected cells which bring about the death of super-sensitive host cells surrounding the infection court. Second, substances are released from these killed host cells which inhibit further development of the pathogen. The sequence of events revealed in this paper on powdery mildew infection of barley varieties might be explained by this hypothesis. Past workers have shown that both permeability changes (9, 10), and increased respiration (1, 2) occur in the mesophyll below the infection court of powdery mildew infected wheat. Evidence that sub-

[4] Verbally.

stances may be released from injured or killed host cells and inhibit the development of the powdery mildew fungus is revealed in the leaf pressure experiments reported by Yarwood (11). He found that pressing bean leaves at 80 lbs. per square in., 24 hours after inoculation with powdery mildew (*Erysiphe polygoni*) greatly reduced mildew development. In pressure experiments on susceptible barley carried out by the writers, and to be reported elsewhere, increasing pressure from 5 lbs. to 30 lbs. per sq. in. applied to leaves immediately before inoculation with *E. graminis hordei* resulted in a decrease in the amount of infection and development of mildew on the pressed areas.

In the work reported in this paper it was demonstrated that mesophyll collapse beneath the infection courts followed the formation of haustoria. This might be interpreted as the culmination of a rapid alteration of host cell physiology due to the presence of toxins. The rate of alteration, and coincidentally the degree of resistance would depend on the state of sensitivity of the mesophyll cells to the toxin—the less sensitive the mesophyll cells to the toxin, the more toxin required to bring about their collapse. Thus, in highly resistant varieties 1 haustorium is sufficient to produce enough toxin for cell collapse. These collapsed cells in turn appear to prevent any further development of the pathogen, whereas in semi-resistant varieties the cells are much more tolerant to the toxin and a greater concentration is needed to bring about the mesophyll collapse. It takes 6–10 haustoria to produce the same effect in these hosts as in the highly resistant ones. Once the mesophyll collapses further development of the pathogen is inhibited. In susceptible varieties the mesophyll cells tolerate the toxins produced even when there are 25–30 haustoria in each infection court. Since there is not any mesophyll collapse in these hosts, development of the pathogen goes on uninhibited.

FACULTY OF AGRICULTURE
UNIVERSITY OF SYDNEY
SYDNEY, NEW SOUTH WALES
AUSTRALIA

LITERATURE CITED

1. ALLEN, P. J., AND D. R. GODDARD. 1938. A respiratory study of powdery mildew of wheat. Amer. Jour. Bot. **25**: 613-621.
2. ALLEN, P. J., AND D. R. GODDARD. 1938. Changes in wheat metabolism caused by powdery mildew. Science **88**: 192-193.
3. CHEREWICK, W. J. 1944. Studies on the Biology of Erysiphe graminis D.C. Can. Jour. Res. C. **22**: 52-86.
4. CORNER, E. J. H. 1935. Observations on resistance to powdery mildew. New Phytol. **34**: 180-200.
5. MACKIE, J. R. 1928. Localization of resistance to powdery mildew in the barley plant. Phytopathology **18**: 901-910.
6. MAINS, E. B., AND S. M. DEITZ. 1930. Physiologic forms of barley mildew Erysiphe graminis hordei, Marchal. Phytopathology **20**: 229-239.
7. MÜLLER, K. O. 1928. Über die Züchtung Kraütfaulerrestenter Kartoffelsorten. Zschr. f. Pfl. Züchtung **13**: 143-156.
8. SMITH, O. F. 1938. Host-parasite relations in red clover plants resistant and susceptible to powdery mildew Erysiphe polygoni. Jour. Agr. Res. **57**: 671-682.
9. THATCHER, F. S. 1939. Osmotic and permeability relations in the nutrition of fungus parasites. Amer. Jour. Bot. **26**: 449-458.
10. THATCHER, F. S. 1942. Further studies on osmotic and permeability relations in parasitism. Can. Jour. Res. C. **20**: 283-311.
11. YARWOOD, C. E. 1953. Pressure effects in fungus and virus infections. Phytopathology **43**: 70-72.

WILT RESISTANT MUTATIONS IN THE CAYENNE VARIETY OF PINEAPPLE[1]

J. L. Collins and Walter Carter[2]

SUMMARY

The 3 clones selected from a large population of plants in a vegetatively propagated variety show some resistance to an insect-caused disease to which the variety is generally susceptible. The degree of resistance is variable among the 3 clones and within each clone, this variability being affected by at least the 2 factors of mealybug source and repeated infestations with large colonies of mealybugs. When resistant plants show wilt symptoms, the symptoms are frequently extremely mild. Resistance is transmitted to progeny through seed reproduction. The resistance character is believed to have originated through somatic mutations in the asexually propagated variety.

Mealybug wilt affects the Cayenne variety of pineapple in most areas where this variety is grown (3). In Hawaii, the disease became increasingly severe until its cause was determined in 1932 and adequate control developed (1, 2). Resistance to the disease was reported in 1947 (6). This study previously concerned a Cayenne-Queen hybrid. The purpose of the present paper is to report the existence of resistant Cayenne clones and the transmission of this resistance to progeny through seed.

RESISTANCE IN CAYENNE CLONES.—Prior to 1932, many attempts had been made to secure resistance to the disease by selection of individual healthy Cayenne plants in diseased areas and by development of clones

[1] Accepted for publication July 22, 1954.
Published with the approval of the Director as Technical Paper No. 226 of the Pineapple Research Institute, Honolulu, Hawaii.
[2] Geneticist and Entomologist, respectively.

TABLE 1.—*Record of wilt appearing in the original planting which showed resistance in 3 Cayenne clones*

Clone	No. Plants	Percentage wilted
LH20	184	41.3
573	390	3.8
666	203	3.4
1069	270	6.7
Field run	205	52.2
7898[a]	829	5.1

[a] A resistant Cayenne × Queen F_1 hybrid.

from these non-wilting selected plants. Although many thousands of plants were so selected, no resistant clones were located.

In 1942, just 10 years after the cause of the disease had been determined and when a high degree of control had been attained, a natural infestation of mealybugs occurred in an experiment station first ratoon field where a number of Cayenne clones and non-selected field run plants were growing. Wilt developed in this area, but 3 fairly large clones exhibited a lower percentage of diseased plants than the other clones and the field run plants. These 3 clones, 573, 666, and 1069, appeared to be resistant to wilt and Table 1 shows the percentage of wilting plants.

When the records in Table 1 are summarized for the 2 types of populations, the resistant clones show an average of 4.7 per cent wilting plants as contrasted to an average of 46.7 per cent in the susceptible groups of plants, LH20 and field run. Subsequent studies produced additional evidence that these 3 clones were resistant, but not immune, to the wilt disease.

SOME FACTORS AFFECTING RESISTANCE.—When Cayenne plants are experimentally infested with mealybugs (*Pseudococcus brevipes*), seldom do all the plants so treated develop the disease. However, on a reinfestation they may develop wilt in a characteristic manner. Because of this irregular feature of wilt development, reliable information cannot be obtained from infestation of single plants. Consequently, clones have been used as units in testing for resistance and susceptibility.

In tests with the 3 resistant clones, 573, 666, and 1069, occasionally a few plants would wilt as severely as the plants of the susceptible variety. There might be several possible explanations for the presence of these wilting individuals in otherwise resistant clones. A probable one is that they represented plants that were admixtures or contaminations in the clone from the susceptible members of the variety. Such contaminations would not be members of the clone in which they were found. During the long time these clones were maintained as separate units but in close proximity to other groups of plants, ample opportunity had existed for such admixture to take place.

In order to produce large populations of these resistant clones, use was made of the stump section method of vegetative propagation. This method involves cutting the stem (or stump) of a mature plant into 25–30 sections, each having 1 or more dormant axillary buds. These, when planted in the ground (or hot beds), produce small plants. In some of the beds, sections from individual stumps were kept together so that sub-clones were established. When these sub-clones were infested with mealybugs, a few showed wilt in all or most of the plants, while adjacent sub-clones did not wilt. These results indicated a probable admixture of susceptible plants among those of the resistant clones.

TABLE 2.—*Percentage of wilting plants resulting from gross infestations of different clones and field run with P. brevipes from 2 source fields, A and B, on different dates*[a]

Source	Date	7898	1069	666	573	LH20	F.R.[b]
				Crown Plantings			
		%	%	%	%	%	%
A	2/9/44	10	25	15	80	60	100
A	2/9/44	15	60	5	40	75	100
A	5/18/44	10	0	5	15	75	95
A	5/18/44	10	5	0	10	80	90
B	3/31/44	10	27	20	82	60	65
				Slip Plantings			
A	5/18/44	5	72	0	10	62	95
A	5/18/44	2	10	5	5	95	97
B	3/31/44	7	32	0	40	72	37
				Sucker Plantings			
A	5/18/44	0	0	7	0	72	97
A	5/18/44	0	10	2	2	87	75
B	3/31/44	2	32	0	14	37	20

[a] First 4 infestations were of 20 plants each; all others 40 plants.
[b] Field run Cayenne.

All the failure to resist cannot be ascribed to such mixing however, for on occasion, both 1069 and 573 have shown considerable susceptibility. Table 2 records the results from the most extreme case, showing great variability between plots and differential susceptibility to mealybugs from 2 sources on different dates of infestation. 1069 was abandoned following this experiment, but 573 has maintained its resistance in most tests, while 666 has shown the most consistent resistance.

In Table 3 a comparison is made of the percentage of wilt produced by mealybugs from 2 sources, A and B. This comparison brings out rather clearly both a

TABLE 3.—*Average percentage wilt produced by source A and B bugs in 4 clones*

Type Planting Material	Source of bugs	Clones 1069 %	666 %	573 %	LH20 %	F.R. %
Crowns	A	22.5	6.2	38.2	75.5	96
	B	27.0	20.0	82.0	60.0	65
Slips	A	41.0	2.5	7.5	78.5	96
	B	32.0	0	40.0	72.0	37
Suckers	A	5.0	4.5	1.0	79.5	86
	B	32.0	0	14.0	37.0	20

TABLE 4.—*Tests for resistance of 4 Cayenne clones to mealybug wilt showing effect of clone, age of test plant, and a second infestation of mealybugs on wilt incidence*

Section		1				2				3				4			
Infestation date—1942	1st	April 24				June 24				July 24				Aug. 26			
	2nd	July 24				Aug. 26				Sept. 24				Oct. 23			
Clone		LH20	573	666	1069	LH20	573	666	1069	LH20	573	666	1069	LH20	573	666	1069
Number of Plants Infested		50	50	50	50	52	53	54	50	50	47	50	50	50	50	50	25
Cumulative no. of wilted plants on successive dates																	
August 17		34	3	0	2	21	2	0	0								
September 2		34	6	1	2	27	3	0									
September 24		34	8	6	2	28	3	2	0	30	2	3	1		0	0	0
December 3											11		9	10	1	5	2
December 23		38	14	20	11	30	19	5	10	35	19	21	16	11	3	6	2
			(1) [a]	(5)	(1)		(12)	(4)	(9)		(10)	(10)	(14)		(2)	(4)	(2)

[a] Figures in () indicate the number of plants out of wilted total showing wilting on outer leaf tips only.

difference in wilt producing capacity of these 2 mealybug populations and a variation between the clones in respect to degree of wilt susceptibility. This difference in wilt inducing capacity of mealybugs from different fields has been noted before (4). It could perhaps reflect strain differences in mealybugs. A more likely explanation is that host plants differ in value as source plants (5) and these differences may vary from field to field.

Thus, in the crown planting, source A bugs caused considerable wilt in clones 1069 and 573, a larger amount in LH20, but were unable to cause as much wilt in 666. Source B bugs caused considerable wilt in all clones, but wilt was especially high in 573.

The production of wilt in plants derived from slips and from suckers is more variable, but again the B source bugs produced a higher percentage of wilt in 1069 and 573 than did A bugs, except in slips of 1069 where B produced slightly less wilt than the bugs from A source.

Although the appearance of any wilt symptom puts the plants in the wilt column, limited symptom expression is frequently encountered in the resistant clones. This is shown in Table 4. Note that the resistant clones showed a fairly high incidence of wilt only after a second infestation of mealybugs and that the symptoms were of limited expression.

The most positive assurance of resistance in these clones has come from field tests which relied upon natural infestation continued over the normal growth period of the crop. One such experiment is shown in Figure 1, and a natural field infestation of 573 and field run plants in Figure 2.

TESTS FOR THE HEREDITARY BASIS OF WILT RESISTANCE.—The experiments described below were carried out to obtain evidence as to whether this resistance had its origin in some kind of an acquired resistance or to a change in the genotype of these plants. Due to self-incompatibility in the pineapple, self-fertilization of plants, or crossing of plants from different sub-clones is not possible. Accordingly, breeding experiments had to make use of crosses between different varieties. Two of the resistant clones, 673 and 1069, and a susceptible Cayenne clone, LH20, were each crossed with the wilt resistant hybrid variety 7898 (6).

Due to the fact that not all of a group of susceptible plants develop wilt from a single infestation, results from infestation of single F_1 hybrid plants would not be reliable indications of resistance or susceptibility. Consequently, clones from individual F_1 plants were developed before tests for wilt resistance were started. Clones from each of the 3 F_1 populations were grossly infested with mealybugs and the determination of the amount of wilt made 5 months later.

In order to reduce the probability of including susceptible plants (clones) with the resistant classification, a procedure was followed which required a clone to contain at least 5 plants all of them free from wilt in order to be classed as resistant. One wilting plant in any clone would place that clone in the susceptible class. This procedure may have resulted in some of the resistant clones being eliminated because they contained less than 5 plants, and some of the susceptible ones because none of them showed the disease. Classification into resistant and susceptible clones for each of the 3 F_1 populations is shown in Table 5.

In Table 6 the data of 2 F_1 populations (3489 and 3664) from resistant parents are grouped together for comparison with the segregation in the F_1 population

TABLE 5.—*Segregation of resistant and susceptible clones in three F_1 populations*

Lot. No.	Type of cross	No. of clones Resist.	No. of clones Susc.	Per cent Resist.	Total Plts.
3489	Cayenne 573 × hybrid 7898	51	48	51	703
3664	Cayenne 1069 × hybrid 7898	15	7	68	160
3410	Cayenne LH20 × hybrid 7898	97	145	40	1818

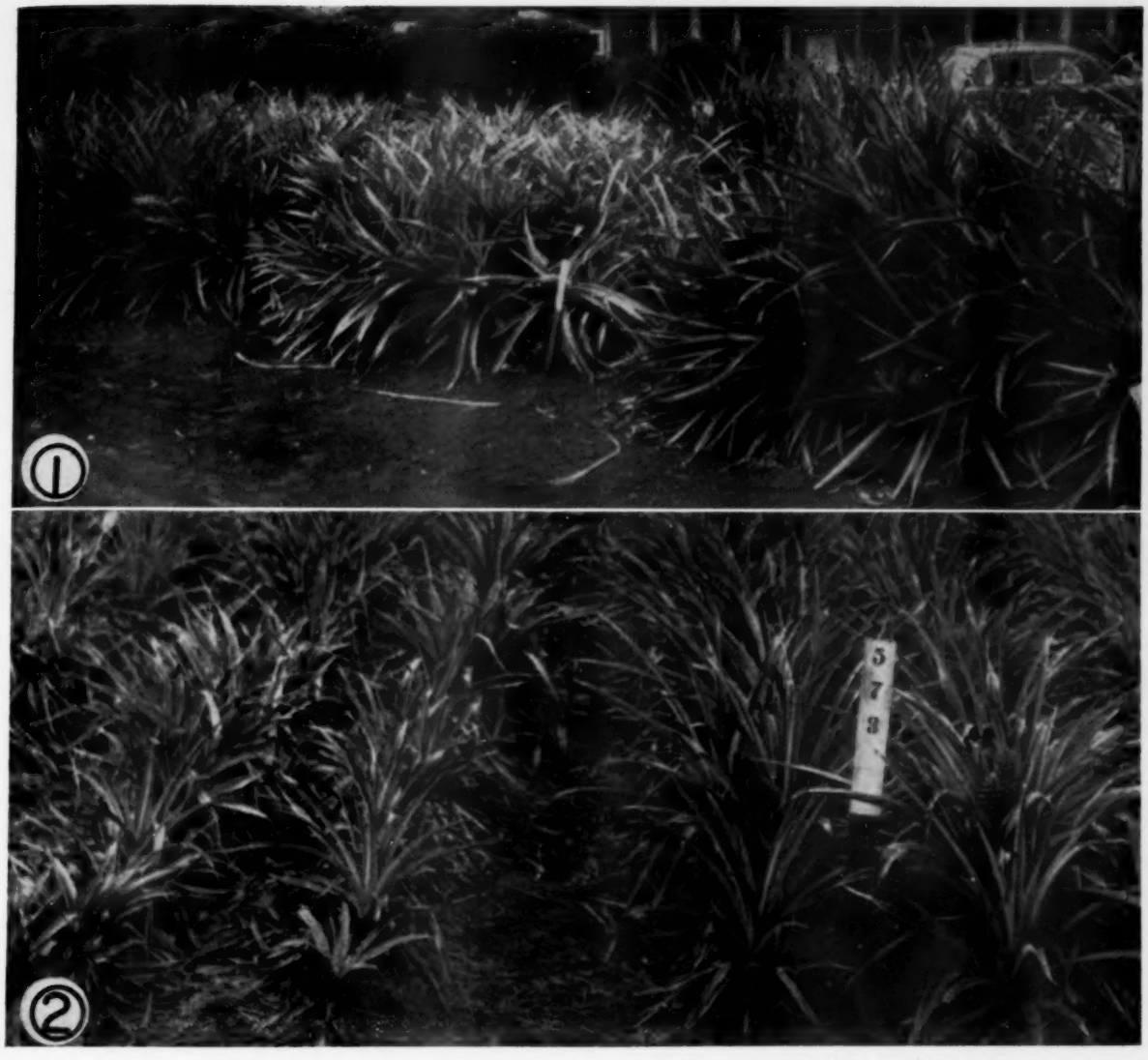


FIG. 1–2. Fig. 1. Plants in first ratoon, 3½ years after planting. No insect control after the first 6 months. All plants became heavily infested with mealybugs. The middle plot is susceptible clone LH20 and the lateral plots a mixture of resistant clones 573 and 666. The susceptible LH20 shows 100 per cent wilt, the resistant clones none. Fig. 2. Plants growing in a plantation field showing wilt resistant clone 573 on the right with wilting field run plants in the bed on the left. Natural mealybug infestation.

(3410) having 1 parent susceptible to wilt.

The calculated numbers in Table 6 are based upon the assumption that the number of resistant and susceptible clones are proportional to the totals in the 2 populations when only random chance variations are operating. The measure of deviation is then obtained by use of the Chi-square (χ^2) method as used by Snedecor (8) where O = the observed and C = the calculated numbers in the formula $(O-C)^2/C$. The value of the Chi-square obtained (6.79) is close to the 1 per cent value for random variation, and indicates a highly significant difference between the 2 populations in regard to the number of wilt susceptible clones produced. Testing for significance of percentage of resistant plants in the 2 populations produces similar results, indicating a highly significant difference. In this analysis (Table 7) the numbers of plants in the clones (instead of the number of clones) is used, thus increasing the size of the populations.

The probability of obtaining a value as large as 6.24 by chance distribution of the 2 classes is so small that it can be concluded that the difference between the 2 populations is due to a genetic difference which existed between the parent plants of the 2 groups.

There is also a question as to whether the susceptible plants in the progeny of resistant parents (573 × 7898 and 1069 × 7898) are different in relation to wilting capacity from the susceptible plants emerging from

TABLE 6.—*The Chi-square analyses of the F_1 segregation into 2 groups, one of which (Lot 3410) produced more susceptible plants than the other group*

No. of	Lot No.	Segregation Observed	Segregation Calculated	$(O-C)^2$ C
Resistant	3489 & 3664	66.0	54.3	2.52
	3410	97.0	108.6	1.23
Susceptible	3489 & 3664	55.0	66.6	2.02
	3410	145.0	133.3	1.02
			Total χ^2 =	6.79

the cross of a susceptible parent (LH20) and the resistant hybrid parent. The Chi-square analyses in Table 8 show that the segregating susceptible clones are alike in the average number of wilting plants per clone regardless of the parentage.

The χ^2 value of 2.88 indicates a lack of significant difference between observed and calculated segregation and, therefore, shows that the susceptible plants are essentially the same whether they segregate in a hybrid population produced by resistant parents or from one produced by a susceptible and a resistant parent.

DISCUSSION.—When resistance to wilt was first observed in these clones, some uncertainty existed as to the nature or origin of the apparent resistance. This was especially true after a systematic infestation of a fairly large number of Cayenne clones and the observations on a larger number of plants naturally infested with mealybugs had failed to indicate resistance within the variety. These clones were not included among those tested for resistance mentioned above; consequently their resistance remained undetected until the accidental mealybug infestation occurred.

The results of these breeding experiments show that resistance is transmitted through seed propagation to progeny and, consequently, leaves little doubt that the resistance originated through somatic mutation. Wilt resistance may now be added to the long list of somatic mutations which have been identified in this vegetatively propagated variety (7). When this

TABLE 7.—*Testing for difference in the percentage of wilting plants in the 2 populations*

Lot Numbers	Plants	Number Resistant	Percentage Resistant	Standard Deviation
3489 } 3664 }	866	415	47.9 ±	2.88
3410	1818	640	35.2 ±	1.25
Difference			12.7 ±	2.03

$$\frac{\text{Difference, } 12.7}{\text{S.E. Difference, } 2.03} = 6.24$$

TABLE 8.—*Comparison of the variability in average number of wilting plants per clone in susceptible clones derived from 2 resistant parents and in susceptible clones derived from a cross where one parent was susceptible and one resistant*

Wilt Classification	Lot Number	Observed	Calculated	$(O-C)^2$ C
Susceptible	3489 & 3664	127	142	1.60
	3410	376	363	0.50
Resistant	3489 & 3664	317	302	0.56
	3410	757	770	0.22
			Total χ^2 =	2.88

mutation occurred is not known, but it probably was present in the variety population from which these clones were started in 1924. The mutation could also have occurred after the clones were established, but the fact that the majority of the plants were resistant argues against this possibility.

The 3 clones, 573, 666 and 1069, are very much alike in obvious characters, but they appear to be unlike in the degree of resistance to wilt. Some evidence is presented showing that bugs collected in different places differ in the ability to produce wilt, suggesting either strain differences among the mealybugs or, more likely, differences in source nature of the plants from which the mealybugs were collected.

It is clear also that field run plants generally are as susceptible as the susceptible clone LH20 which chanced to be planted in the same field with the resistant clones when evidence for resistance was first observed.

PINEAPPLE RESEARCH INSTITUTE
HONOLULU, HAWAII

LITERATURE CITED

1. CARTER, W. 1933. The pineapple mealybug Pseudococcus brevipes and wilt of pineapple. Phytopathology **23**: 207-242.
2. CARTER, W. 1935. Diesel oil emulsions as insecticides. Jour. Econ. Ent. **28**: 268-284.
3. CARTER, W. 1942. The geographical distribution of mealybug wilt with notes on some other insect pests of pineapple. Jour. Econ. Ent. **35**: 10-15.
4. CARTER, W. 1951. The feeding sequence of Pseudococcus brevipes (Ckl.) in relation to mealybug wilt of pineapples in Hawaii. Phytopathology **51**: 769-780.
5. CARTER, W. 1952. The injuries to plants caused by insect toxins. II. Bot. Rev. **18**: 680-721.
6. CARTER, W., AND J. L. COLLINS. 1947. Resistance to mealybug wilt of pineapple with special reference to Cayenne-Queen hybrid. Phytopathology **37**: 332-348.
7. COLLINS, J. L., AND K. R. KERNS. 1938. Mutations in the pineapple. Jour. Hered. **29**: 162-172.
8. SNEDCOR, G. W. 1938. Statistical methods. Ed. 2. Collegiate Press Inc., Ames, Iowa.